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CARDIAC LESIONS ASSOCIATED WITH CHRONIC INFECTIOUS ARTHRITIS

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Opinions as to the presence and nature of cardiac lesions associated with chronic infectious (rheumatoid) arthritis have been varied. Fuller [1] and Garrod [2] have stated that, as a rule, the heart is not injured in rheumatoid arthritis. Fuller stated that he had never seen a case in which he could attribute pericarditis or endocarditis to rheumatoid arthritis. Garrod observed that if patients with rheumatoid arthritis have valvular insufficiency, one can assume a previous attack of rheumatic fever has been the cause of the lesion.

Strümpell [3] and Pribram [4] stated that in rheumatoid arthritis of slow, insidious onset cardiac involvement does not occur, but when the disease is ushered in by an acute febrile attack of arthritis the heart is often implicated.

Barjon,[5] on the other hand, estimated that the number of cardiac complications is just as high in the cases of rheumatoid arthritis with chronic onset as in those of acute polyarthritis at the onset.

Clinical investigations of the heart in cases of chronic infectious arthritis have been reported by Bannatyne,[6] Barjon,[5] Pribram,[4] McCrae,[7]

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1. Fuller, cited by Pribram.[4]

2. Garrod, A. E.: A Treatise on Rheumatism and Rheumatoid Arthritis, London, Charles Griffin and Company, 1890, pp. 261-263.

3. Strümpell, A.: Lehrbuch der speciellen Pathologie und Therapie der inneren Krankheiten für Studierende und Aerzte, Leipzig, F. C. W. Vogel, 1902, vol. 3, pp. 525-526.

4. Pribram, A.: Chronischer Gelenkrheumatismus und Osteoarthritis deformans, in Nothnagel, H.: Specielle Pathologie und Therapie, Vienna, A. Hölder, 1902, vol. 7, pt. 5, p. 62.

5. Barjon, cited by Boas and Rifkin.[8]

(Footnotes continued on next page)

Boas and Rifkin,[8] Froggatt,[9] Coates,[10] Master and Jaffe,[11] Kahlmeter,[12] Monroe and Walcott,[13] and Monroe.[14] According to these reports, the clinical incidence of cardiac lesions varies from 4 per cent (Coates and Monroe) to 40 per cent (Kahlmeter, for "secondary chronic arthritis").

The value of these clinical reports is limited because (1) the type of chronic arthritis studied is not always clearly defined, (2) clinical data (symptoms, signs, roentgenograms and electrocardiographic tracings) are often insufficient to make an accurate diagnosis of the nature or site of a cardiac lesion and (3) mild or healed cardiac lesions may give no clinical indication whatsoever of their presence.

Pathologic studies of the heart in cases in which chronic infectious arthritis is known to have occurred during the life of the patient are few. Charcot[15] mentioned that there was pericarditis in 4 out of 9 cases of "chronic rheumatism" in which necropsy was performed. Kast,[16] in 1901, studied the postmortem incidence of cardiac lesions in cases in which "chronic rheumatism" of various types had occurred during life. Of 13 cases of "primary chronic arthritis" (rheumatoid?), endocarditis occurred in 2, subacute pericarditis in 1 and obliteration of the pericardial sac in 1. In 3 of 4 cases of "secondary chronic arthritis," endocarditis

6. Bannatyne, G. A.: Rheumatoid Arthritis: Its Pathology, Morbid Anatomy and Treatment, ed. 2, Bristol, John Wright and Company, 1898, pp. 115-116.

7. McCrae, T.: Arthritis Deformans, in Osler, W., and McCrae, T.: Modern Medicine: Its Theory and Practice, ed. 3, Philadelphia, Lea & Febiger, 1915, vol. 5, pp. 914-915.

8. Boas, E. P., and Rifkin, P.: The Heart in Arthritis Deformans, J. A. M. A. **82**:1596-1599 (May 17) 1924.

9. Froggatt, T. W.: Incidence of Heart Lesions in Infective Arthritis, Lancet **2**:116 (July 19) 1924.

10. Coates, V.: The Kinship of Rheumatic Fever and Rheumatoid Arthritis, M. J. & Rec. **133**:55-56 (Jan. 21) 1931.

11. Master, A. M., and Jaffe, H.: Rheumatoid (Infectious) Arthritis and Acute Rheumatic Fever: The Differential Diagnosis, J. A. M. A. **98**:881-882 (March 12) 1932.

12. Kahlmeter, G.: De l'existence de lésions myocardiques et valvulaires dans les diverses formes de polyarthrites chroniques et des conclusions qu'on en peut tirer touchant l'étiologie et le groupement clinique des polyarthrites chroniques, Acta med. Scandinav., 1934, supp. 59, pp. 611-625.

13. Monroe, R. T., and Walcott, C. F.: The Incidence of Cardiovascular Disease in Chronic Arthritis, in Medical Papers Dedicated to Henry Asbury Christian, Physician and Teacher, in Honor of His Sixtieth Birthday, Baltimore, Waverly Press, Inc., 1936, pp. 918-927.

14. Monroe, R. T.: Chronic Arthritis, in Christian, H. A.: Oxford Medicine, New York, Oxford University Press, 1936, vol. 4, pt. 2, pp. 307-404.

15. Charcot, J. M.: Clinical Lectures on Senile and Chronic Diseases, translated by W. S. Tuke, London, The New Sydenham Society, 1881, pp. 172-175.

16. Kast, L.: Ueber das Verhalten der Herzaffectionen bei chronischen Gelenkrheumatis, resp. Arthritis deformans, Prag. med. Wchnschr. **26**:493-494, 508-509, 521-523 and 531-533, 1901.

occurred. Kast expressed the belief that there is a fundamental difference between those instances of acute onset (secondary chronic arthritis), in 75 per cent of which there was endocarditis, and those of insidious onset (primary chronic arthritis), in only 30.8 per cent of which endocarditis or pericarditis occurred.

Grzimek,[17] in 1932, found that in 39 (42.8 per cent) out of 91 cases of "genuine arthritis deformans" (rheumatoid?) recurrent or healed valvular disease was present. He included in his study only cases in which exostoses (*Randwucherungen*) were present on joint margins. He excluded all cases of "genuine arthritis deformans" in which acute endocarditis was present. Because of this exclusion and the fact that the type of arthritis that he studied was not clearly defined, his results are difficult to interpret.

The relative lack of careful anatomic studies of the heart in cases of chronic infectious (rheumatoid) arthritis is in contrast to the large number of such studies in cases of rheumatic fever. Therefore, the present study was undertaken. We also hoped that such an investigation might throw light on the relationship between rheumatic fever and chronic infectious arthritis.

MATERIAL AND METHODS

This investigation was based on a study of the necropsies in 25 cases of chronic infectious (rheumatoid) arthritis, in only 2 of which there was a history of rheumatic fever. The pathologic material at the Mayo Clinic was surveyed, and all cases were included in which the following clinical criteria in regard to the joint disease had been fulfilled: All patients whose cases were included in this study had polyarticular inflammation; generally the onset had been insidious, although occasionally the beginning had been explosive. In each instance, the arthritis had run a progressive course for months or years. There was always some degree of articular crippling. In some, the joint disease had become relatively inactive but it had always caused changes which were easily detectable on clinical examination. Many of the involved joints had a spindle-shaped appearance because of swelling of the joint accompanied by atrophy of the adjacent muscles. Periarticular and synovial thickening were common, and frequently effusions were present. Some degree of fibrous ankylosis was a usual finding.

Roentgenograms were frequently normal in early stages, although in well developed stages there were swelling of periarticular soft parts, narrowing of the joint space, atrophy of epiphysial bone, destruction of subchondral bone and, later, marginal lipping.

Systemic manifestations were usually present; these frequently included slight fever and loss of weight associated with some degree of hypochromic anemia. The concentration of hemoglobin averaged 66.6 per cent in these cases. The number of erythrocytes per cubic millimeter of blood averaged approximately 3,990,000.

In each instance, the diagnosis of rheumatoid arthritis had been made by a consultant of the Section on Arthritis of the Mayo Clinic.

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17. Grzimek, N.: Ueber die Häufigkeit des Zusammentreffens von Arthritis deformans und chronischer Endokarditis, Virchows Arch. f. path. Anat. **286**:286-290, 1932.

TABLE 1.—*Clinical Data and Necropsy Observations on the Heart of Twenty-Five Cases of Chronic Infectious Arthritis*

Case No. and Sex	Age, Years: At Onset of Arthritis	Age, Years: At Death	Clinical Findings in Joints	Report of Roentgenograms of Joints	Hemoglobin, Red Blood Cells	Pathologic Diagnosis of Heart Lesion
1 M	48	55	Shoulder movements limited, 3; flexion deformity of right elbow, 45 degrees; atrophy of interosseous muscles of hands; spindle-shaped swellings of midphalangeal joints of fingers; flexion deformities of right knee, 30 degrees, and left knee, 60 degrees	Hypertrophic changes around knee joints	70% 4,240,000	Subacute rheumatic mitral and aortic valvulitis; coronary arteritis; acute (focal and diffuse) myocarditis
2 M	11	17	Swollen knees held in flexion deformity of 80 degrees; spindle-shaped swellings of midphalangeal joints of hands; atrophy of interosseous muscles of hands; shoulder movements limited, 2; ankylosis of wrists and elbows; swollen and rigid feet	Knees markedly swollen; bones osteoporotic	50% 3,500,000	Subacute rheumatic pericarditis with beginning organization; acute and chronic focal myocarditis
3 F	22	24	Duration of arthritis 25 months; spindle-shaped swellings of midphalangeal joints of fingers; elbows, wrists, knees and ankles swollen and motion limited; flexion deformities of elbows and knees; extreme atrophy of muscles adjoining affected joints, characteristic of chronic infectious arthritis; spondylitis present	Evidence of atrophic arthritis of both hands	65% 4,210,000	Subacute rheumatic mitral endocarditis; fibrinous pericarditis; diffuse and nodular myocarditis, and coronary arteritis
4 M	42	48	Shoulder motions painful; flexion deformity of left elbow 5 degrees, and right elbow 10 degrees; left wrist swollen and tender; knuckles swollen and tender; hip motions limited, right 1 and left 1+; right knee swollen; 2, fluid present; left knee swollen, 1+; metatarsal joints tender	Narrowing of joint spaces, with swelling of soft tissues and osteoporosis of knees, left hand and right wrist and foot	 3,710,000	Chronic active rheumatic mitral endocarditis with noninfected thrombus attached; acute and chronic rheumatic and focal aortitis
5 F	22	24	Jaw movements limited, 2; swelling of proximal, interphalangeal and metacarpophalangeal joints; elbows limited, 1; movements of both shoulders limited; knees and feet stiff	Periarticular swelling of joints of hands	92.7% 4,500,000	Chronic rheumatic adhesive pericarditis with subacute exacerbation
6 F	45	57	A wheel-chair invalid because of pain, heat and swelling of many joints; flexion deformities of knees; spondylitis present	Marked destructive arthritis of knees and hands accompanied by ankylosis	70% 4,010,000	Active chronic rheumatic endocarditis of mitral, aortic and tricuspid valves; aortitis and pericarditis; acute pulmonary endocarditis; chronic diffuse and focal myocarditis (mitral stenosis, grade 2)
7 F	30	49	Deformities of joints of hands and feet characteristic of chronic infectious arthritis; subluxation and adduction of fingers	.....	....	Acute exacerbation of chronic rheumatic obliterative pericarditis; acute focal myocarditis; acute coronary periarteritis, chronic healed (?) mitral endocarditis
8 F	12	14	Jaws and all large joints limited; knees and ankles almost completely ankylosed	.....	....	Chronic active rheumatic mitral endocarditis and myocarditis; chronic aortic and tricuspid endocarditis
9 F	38	42	Motion of jaws limited; left shoulder and wrists with limited motion; slight flexion deformities of elbows; spindle-shaped swellings of midphalangeal joints; metatarsal joints tender	Evidence of atrophic arthritis in wrists and fingers	40% 3,420,000	Chronic rheumatic mitral endocarditis and healed rheumatic myocarditis (mitral stenosis, grade 2+)
10	60	63	Rheumatoid arthritis with "horrible deformities"; activity of inflammatory process only slight	Soft tissue swelling of knees with flexion deformities	53%	Chronic rheumatic mitral and aortic endocarditis with calcification; healed rheumatic myocarditis (aortic stenosis, grade 1)
11	52	52	Duration of arthritis 5 months; midphalangeal joints	Normal	97.3%	Inactive rheumatic mitral endocarditis with

11 M	53	52	Duration of arthritis 5 months; midphalangeal joints of right hand swollen; swollen tender metatarsal joints	Normal	97.3% 4,100,000	Inactive rheumatic mitral endocarditis with an organizing thrombus
12 F	46	49	Right ankle, midphalangeal joints and left wrist swollen and red	Swelling of soft tissues and destruction of cartilage of right ankle	93.3% 4,130,000	Inactive rheumatic mitral and aortic endocarditis with stenosis of mitral valve (mitral stenosis, grade 3)
13 F	30	53	Shoulder motions limited; flexion deformities of knees, right 15 degrees, left 20 degrees; right wrist limited; atrophy of interosseous muscles of hands with hyperextension deformities of midphalangeal joints of hands; limitation of motion in right hip	Destructive type of arthritis of right knee with marked periarticular swelling	70% 4,520,000	Inactive rheumatic mitral endocarditis (mitral stenosis, grade 3)
14 M	32	38	Limitation of motion of elbows, wrists and ankles; swollen midphalangeal joints of hands; flexion deformities of knees	Ankylosis of knees	25% 2,740,000	Inactive rheumatic mitral endocarditis; acute focal myocarditis (rheumatic?)
15 F	?	32	Flexion deformities of elbows and knees; limited motion of fingers, wrists, elbows, shoulders, knees and ankles; knees swollen and boggy; ulnar deviation of fingers; spondylitis	Hypertrophic changes in knee joints	60% 4,000,000	Thrombosis of right coronary artery; old infarct of myocardium
16 M	51	51	Hips limited, 1; right knee swollen.....	Destructive arthritis in knee joints	65% 4,150,000	Nonspecific pericarditis
17 M	35	37	Spindle-shaped swellings of midphalangeal joints of hands; flexion deformities of elbows; pain on abduction of shoulders; painful feet	Hands normal	58% 4,700,000	Hypertrophy of heart, 440 Gm. (hypertension)
18 M	21	34	Extensive spondylitis; thoracic expansion reduced...	Destructive arthritis of both sacroiliac joints and 3d and 4th lumbar vertebrae	110%	Old infarct of myocardium
19 M	47	47	Swelling of proximal and midphalangeal joints of hands with atrophy of interosseous muscles and inability to close fists; right elbow, neck and shoulders limited; thoracic expansion, 1 inch (2.5 cm.)	Periarticular swelling of joints of hands with osteoporosis due to atrophic arthritis	70% 4,100,000	Hydropericardium (300 cc.)
20 M	7	26	Flexion deformities of hips and knees; ankylosis of shoulders, elbows and wrists	Destructive arthritis of right knee with ankylosis of right shoulder, elbow, wrist and hand	....	Obliterative fibrous pericarditis (rheumatic?)
21 M	55	55	Characteristic deformities of chronic infectious arthritis in joints of fingers, elbows and feet	.....	....	Normal
22 F	39	69	Ankylosis of wrists and fingers; flexion deformities of knees, 15 degrees	Marked hypertrophic changes in both knees, with destruction of cartilage	95.3% 4,190,000	Normal
23 F	26	30	Multiple ankylosis and contractures; only 30 degrees of motion in left elbow; left wrist ankylosed; contractures of finger joints	Destruction of cartilage of knee joint and fixation of patella due to chronic infectious arthritis	70% 4,360,000	Normal
24 M	?	47	Lumbar and dorsal portion of spine fixed because of spondylitis	Normal	....	Normal
25 M	19	22	Spondylitis with 90 per cent fixation of spine; atrophy of interosseous muscles of hands; swollen wrists and metacarpophalangeal joints; spindle-shaped swellings of midphalangeal joints; flexion deformities right elbow, 20 degrees, left, 30 degrees; only 20 degree motion in hips and knees	Synovial swelling in knees associated with some ankylosis	45% 3,230,000	Normal

After careful gross examination at the time of necropsy the hearts were fixed in modified Kaiserling's [18] solution and then were preserved in neutral solution of formaldehyde U. S. P. Sections for histologic study were taken of all structures that presented abnormal appearances. In addition, routine sections were taken from the mitral and aortic valves and valve rings, from the anterior wall and apex of the left ventricle, as well as from the posterior base of the septum and from the posterior wall of the left auricle. The sections were stained routinely with hematoxylin and eosin. The Van Gieson, Weigert fibrin and Gram stains and the Pappenheim pyronine and de Galantha [19] silver stains were employed when indicated.

RESULTS

The results of this study have been summarized in table 1. There were 20 cases of chronic infectious arthritis with which cardiac damage was associated. Lesions indistinguishable from those produced by rheumatic fever were found in 14, and nonrheumatic lesions were present in 5. In 1 case there was fibrous obliteration of the pericardial cavity, but the nature of this lesion could not be determined because neither the heart nor the histologic sections had been preserved. A normal heart was found in only 5 instances.

Lesions not attributable to rheumatic fever were as follows: coronary sclerosis with thrombosis and acute and chronic infarction of the myocardium (case 15), nonspecific subacute fibrinous pericarditis (case 16), hypertrophy of the heart as a result of hypertension (case 17), coronary sclerosis with chronic infarction of the myocardium (case 18), hydropericardium (case 19) and obliteration of the pericardial sac (case 20).

GROSS APPEARANCE OF RHEUMATIC LESIONS

In all but 1 (case 1) of the 14 cases in which rheumatic lesions were demonstrated in the heart, the lesions had been diagnosed at the time of necropsy from the gross appearance.

Gross lesions were present in the mitral valve in 10 cases. In 5 (cases 3, 9, 11, 13 and 14) the mitral valve was the only one involved; in 3 (cases 4, 10 and 12) both the mitral and the aortic valve were involved, and in 2 (cases 6 and 8) the mitral, aortic and tricuspid valves were involved.

The changes present consisted of variable degrees of diffuse thickening of the leaflets of the mitral valve with shortening and thickening of the chordae tendineae. Verrucae were present in only 5 cases (3, 6, 8, 9 and 14), and in 2 instances uninfected thrombi were attached to the leaflet (cases 4 and 11). Gross evidence of calcification was present in only 1 instance (case 13). In several instances vascularization of the leaflet was grossly apparent.

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18. Robertson, H. E., and Lundquist, R.: The Preparation of Pathologic Specimens for Exhibition Purposes, J. Tech. Methods **13**:31-32, 1934.

19. de Galantha, E.: Personal communication to the authors.

Fig. 1 (case 13).—Thickening of the mitral leaflet with thickening and shortening of the chordae tendineae. Dilatation of the left auricle.

Fig. 2 (case 2).—Subacute fibrinous pericarditis.

These changes had resulted in severe stenosis of the mitral valve in cases 12 and 13 (fig. 1) and moderate stenosis in 2 others (cases 6 and 9). The left auricle was dilated in these 4 cases, and in 1 instance (case 12) there was a mural thrombus in the left auricular appendage.

The aortic valve revealed gross evidence of injury in 5 cases. Thickening and stiffening of the leaflets occurred in 2 cases (6 and 8); adhesions between the cusps at the commissures were present in 2 (cases 4 and 10), and in 1 instance (case 6) there was rounding of the free edge with consequent shortening of the leaflet. Mild stenosis was present with nodular calcification of the leaflets in a single instance (case 10). The leaflets of the tricuspid valve were irregularly thickened in 2 cases (6 and 8). The pulmonary valve revealed no gross indication of valvulitis.

The pericardium was involved in 5 cases. In 3 of these (cases 2, 5 and 7) the pericardium was the only structure that revealed gross evidence of injury, whereas in 2 others (cases 3 and 6) pericarditis was accompanied by rheumatic valvulitis and aortitis. In 3 instances (cases 2, 3 and 5) the pericardial exudate was predominantly fibrinous (fig. 2); in 1 instance (case 6) it was fibrinopurulent, and in another (case 7) there was obliteration of the pericardial sac by fibrous adhesions and calcified masses were present in the obliterated sac.

Chronic infarction was present in case 7 and perivascular scars occurred in case 14. These were the only cases in which there was macroscopic evidence of myocardial disease. Verrucae on the endocardium of the left auricle were present in only 1 instance (case 6).

The mean weight of the hearts in which rheumatic lesions were demonstrated was 379.7 Gm. The mean age of these patients was 42.3 years. The largest heart (case 6) weighed 547 Gm., and the smallest (case 4), 260 Gm. The weights varied between 300 and 400 Gm. in 7 cases and were more than 400 Gm. in 4 cases. The mean weight of the hearts in 5 cases in which nonrheumatic lesions were demonstrated was 386 Gm. The largest heart in this group weighed 620 Gm. (case 16), and the smallest, 263 Gm. (case 19). The mean weight of the hearts without lesions was 249.6 Gm.; the largest weighed 280 Gm. (case 21), and the smallest, 222 Gm. (case 25). It is apparent that in the cases in which there are associated cardiac lesions the mean weight of the heart is higher than is generally accepted as normal and in those in which there are no associated cardiac lesions the mean weight is slightly less than normal.

HISTOLOGIC FEATURES OF RHEUMATIC LESIONS

For convenience of description and discussion, the cases studied may be separated into groups distinguished by the presence of (1) subacute, (2) chronic and (3) inactive lesions. There were 3 instances of subacute, 7 instances of chronic active and 4 instances of inactive rheumatic

lesions in the heart. This does not mean, however, that all the lesions in a single group of cases were of the same type. It simply means that the predominant type of inflammation in any single case could histologically be classified as subacute, chronic or inactive. In group 2, for example, there were many instances, as will be emphasized subsequently, in which evidence of acute or subacute inflammation was found in addition to the chronic inflammation. In table 2, the lesions have also been classified as to their respective sites.

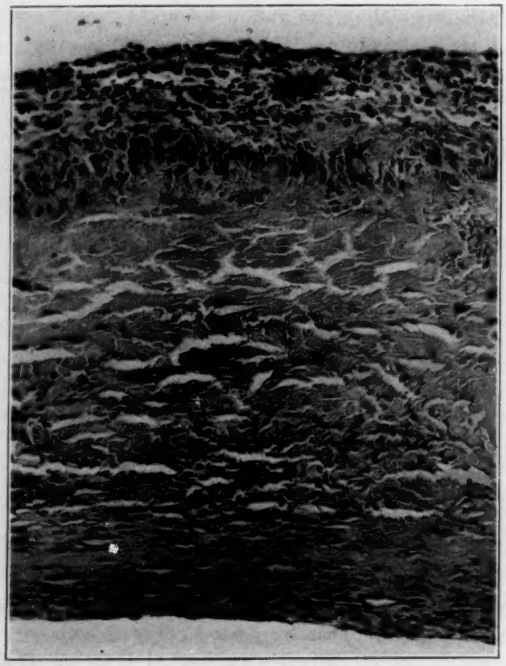

Fig. 3 (case 1).—Subacute aortic rheumatic valvulitis. Palisading of the basophilic cells around a region of fibrinoid degeneration. Hematoxylin and eosin; × 200.

TABLE 2.—*Sites of Rheumatic Lesions*

Structures Involved	Number of Cases
Mitral valve only	6
Mitral and aortic valves	3
Mitral, aortic and tricuspid valves	1
Mitral, aortic, tricuspid and pulmonary valves	1
Pericardium	5
Myocardium	12
Left auricle	3
Root of aorta	3
Coronary arteries	3

Subacute Lesions.—In this group are cases 1, 2 and 3. The valves involved were the mitral and the aortic in case 1 and only the mitral in case 3. In each instance there was severe valvulitis of the entire leaflet with involvement of the valve rings as well. The lesions were typical of those seen in valves in cases of rheumatic fever. Regions of fibrinoid degeneration with large basophilic histiocytes showing a tendency toward a palisaded arrangement were present (fig. 3).

Diffuse fibrinous pericarditis occurred in 2 instances (cases 2 and 3). Myocarditis was present in all 3 cases of this group. The inflamnation of the heart muscle was both diffuse and nodular. In cases 2 and 3 evidence of chronic as well as acute and subacute myocarditis was present. In both of these cases, polymorphonuclear cells were numerous in many of the myocardial lesions and in some regions there were small abscesses. Typical Aschoff bodies were present in all of these cases.

Coronary arteries were involved in cases 1 and 3. In the former the entire wall of the right coronary artery was the seat of subacute inflammation, whereas in the latter only the smaller branches were involved, apparently by extension from the perivascular lesions.

It is interesting to note that in case 1 macroscopic examination of the heart had revealed no lesions, whereas extensive damage was found on microscopic examination.

Chronic and Active Lesions.—In this group of 7 cases, there were 2 (cases 4 and 9) in which only the mitral valve was involved, 1 (case 10) in which both the mitral and the aortic valve were involved, 1 (case 8) in which the mitral, aortic and tricuspid valves were involved and 1 (case 6) in which all the valves were involved.

The most pronounced valvular changes in this group consisted of thickening and vascularization of the mitral leaflets. The thickening was due to an increase of hyalinized collagenous connective tissue generally present throughout the valve, but found especially in the auricularis layer of the auriculoventricular valves. The spongiosa was also frequently replaced by this dense hyalinized connective tissue. Vascularization occurred in the mitral valve in 3 cases (cases 7, 8 and 9), the vessels being thick-walled arteries. In addition to these evidences of a former inflammatory process, there were foci of hystiocytic and fibroblastic proliferation in various portions of the leaflet (fig. 4 *a*). In 3 cases of this group (cases 6, 8 and 9) there were Aschoff bodies in the mitral valve. Small, irregular fibrous nodules were present on the surface of the mitral leaflets in 4 cases (cases 6, 7, 8 and 9). These probably represented organized verrucae.

Lesions were noted in the ring of the mitral valve in 4 cases of this group. Abnormal vascularization was present in 3 (cases 6, 9 and 10), and in 1 (case 6) there were collections of inflammatory cells also. Fibrous polypoid projections from the subvalvular angle of the mitral leaflet occurred in 2 instances (cases 6 and 9).

The aortic valve was less frequently involved, but the histologic changes were similar to those seen in the mitral valve. Thickening of the leaflets was prominent especially in the ventricularis layer. Aschoff bodies occurred in the leaflet in only 1 instance (case 6). Abnormal vascularization in the aortic ring was present in 2 instances (cases 4 and 10), and inflammation was present in 1 (case 6).

In this group in which chronic active lesions were present, the pericardium was involved in 3 instances (cases 5, 6 and 7). In addition to fibrous adhesions between the parietal and the visceral layer, there were regions of fibrinous exudate with polymorphonuclear cells and collections of proliferating histiocytes. The latter were large and contained baso-

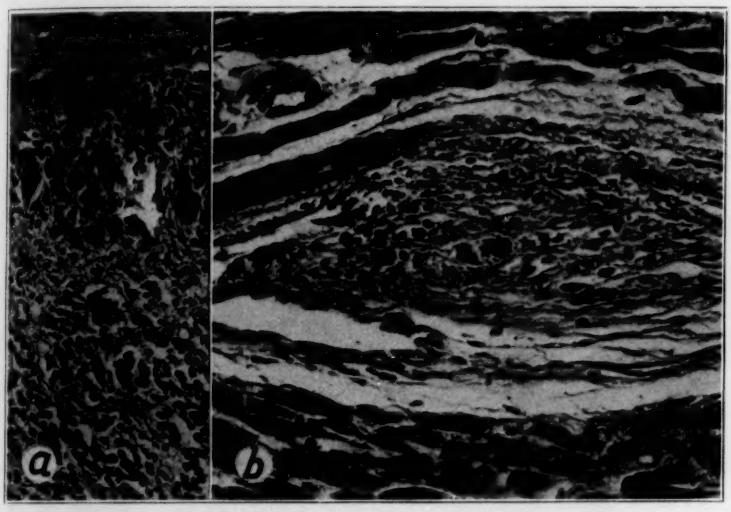

Fig. 4.—Chronic rheumatic lesions. (*a*) Rheumatic mitral valvulitis (case 6). Large basophilic and giant cells are arranged about a region of necrosis. Hematoxylin and eosin; × 195. (*b*) Myocardial Aschoff body (case 8). Hematoxylin and eosin; × 245.

philic cytoplasm and large pale nuclei. Occasionally giant cells were noted. The cells had a tendency to be arranged in palisades.

Lesions were present in the myocardium in each case of this group. In 2 instances (cases 9 and 10) the only evidence of myocardial injury was the presence of perivascular "onion skin" scars. In 2 cases the myocardium appeared to be involved by extension, in 1 (case 4) from an aortic lesion and in the other (case 5) from a pericardial lesion. In 3 other instances (cases 6, 7 and 8) typical Aschoff bodies were present (fig. 4 *b*), while in 1 instance (case 6) there was also diffuse myocarditis.

The left auricle was the site of injury in 3 cases of this group in which chronic active lesions were present. In 1 (case 6) there were mural endocardial verrucae as well as auricular myocarditis; in 1 (case 7) there was auricular myocarditis only, and in 1 (case 4) there was thickening of the endocardium due to fibrous and elastic tissue.

Nonspecific lesions in the coronary arteries were present in 3 cases of this second group (cases 5, 6 and 10) and consisted of organized mural thrombi in the smaller branches. In 1 case (case 10) this lesion was associated with chronic infarction of the myocardium. Rheumatic periarteritis occurred in case 7. The aorta was the site of granulomatous lesions in 2 cases (cases 4 and 6), and typical Aschoff bodies were

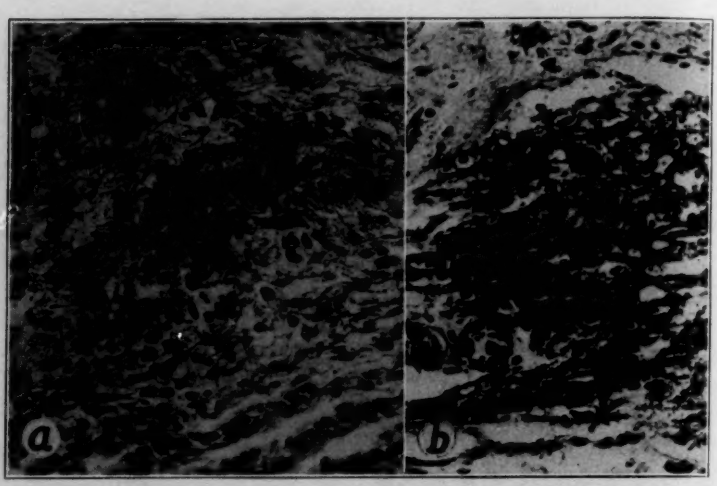

Fig. 5.—Fibrinoid degeneration in cases with associated chronic rheumatic lesions. (*a*) Region of fibrinoid degeneration and proliferation of histiocytes in adventitia of aorta (case 4). Hematoxylin and eosin; × 205. (*b*) Region of acute fibrinoid degeneration in otherwise chronic pericarditis (case 7). Hematoxylin and eosin; × 225.

present in both. Deposits of calcium, either in the mitral or in the aortic valve or in the pericardium, occurred in 4 cases of this group (cases 6, 7, 9 and 10). It is significant that typical Aschoff bodies, in the valves, the pericardium or the myocardium, were found in all of the cases of this group in which chronic active lesions were present, except in cases 9 and 10.

A significant feature of the cases of this group also was the fact that in 5 of them evidence of recent acute rheumatic injury was found in addition to the chronic inflammation. The acute lesions were in some instances superimposed on inactive or chronic inflammatory processes,

and in other instances they appeared in a previously uninvolved region of the heart. Thus, in case 4 chronic mitral endocarditis was associated with regions of "fibrinoid" degeneration and proliferative inflammation in the aorta and myocardium (fig. 5). In case 5 the pericardial sac was obliterated by fibrous adhesions, yet lesions with fibrinous exudate and proliferating histiocytes in both epicardium and myocardium were also found. In case 6 chronic but extremely active lesions in the mitral and the aortic valves, the pericardium and the aorta were accompanied by acute endocarditis of the pulmonary valve. In case 7 there was an acute recurrence of chronic adhesive pericarditis in addition to acute lesions of the myocardium and the coronary arteries (fig. 5). In case 8

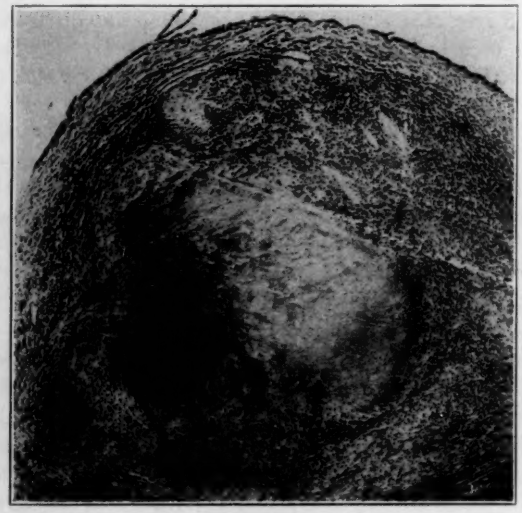

Fig. 6 (case 6).—Chronic "rheumatic" inflammation of the aortic valve with a large central region of necrosis surrounded by proliferating histiocytes and fibroblasts. Hematoxylin and eosin; × 32.

a region of acute "fibrinoid" degeneration occurred in the spongiosa of the mitral valve. This valve had been already thickened by chronic proliferative inflammation.

One case (case 6) in this second group deserves special mention in that the lesions were in many respects similar to those seen in the subcutaneous nodules of chronic infectious (rheumatoid) arthritis. In the pericardium, in the mitral and the aortic valves and in the aorta there were massive proliferations of large histiocytes containing basophilic cytoplasm arranged radially around large central regions of necrosis (fig. 6). Many giant cells were present, and more or less typical Aschoff

bodies could also be seen. The myocardium was the seat of diffuse interstitial myocarditis, but an occasional Aschoff body could also be found. Search was made for Spirochaeta pallida and for tubercle bacilli in specially stained sections, but none were found.

Inactive Lesions.—In this group were 4 cases (cases 11, 12, 13 and 14) in which the macroscopic lesions were found on histologic examination to be burned out or inactive. The mitral valve was involved in all of these, and in 1 instance (case 12) the aortic valve was also injured. The valvular lesions consisted of changes similar to those described for the second group except that no evidence of activity such as regions of fibrinoid degeneration or granulomatous foci could be found. Vascularization of the valve, with thick-walled vessels, was present in 3 instances (cases 12, 13 and 14). Vascularization of the valve rings occurred in 2 instances (cases 11 and 12). Deposits of calcium were found in the mitral valve in 2 instances (cases 11 and 13) in this group.

The ventricular myocardium and septum revealed lesions in 2 cases. In 1 (case 12) the only sign of myocardial damage was the presence of perivascular "onion skin" scars, whereas in the other (case 14) there were scattered foci of interstitial proliferative inflammation but typical Aschoff bodies were not present. Examination of the pericardium, the left auricle and the coronary arteries revealed no lesions in the cases of this group.

CLINICAL CONSIDERATIONS

The cases of 14 male and 11 female patients were included in this study. The mean age was 41 years. Clinically, the joint disease showed some evidences of activity in all cases. Some workers would distinguish between chronic infectious arthritis with an acute onset and chronic infectious arthritis with an insidious onset. Pribram, Kast and Kahlmeter, as noted previously, have found a higher incidence of cardiac damage in those cases in which an acute onset occurred. In all but 3 of our series (cases 3, 16 and 20) the onset of the disease was insidious. Subacute rheumatic lesions were found in the pericardium, the mitral valve and the myocardium in case 3, nonspecific subacute pericarditis was present in case 16 and obliteration of the pericardial sac occurred in case 20. The specimen in the last case, unfortunately, had not been saved, and consequently its rheumatic or nonrheumatic nature could not be determined.

It is of interest to examine our material in the light of Garrod's contention that the occurrence of cardiac lesions among patients who have chronic arthritis can be attributed to a previous attack of rheumatic fever. In the present series, a history of rheumatic fever could be elicited in only 2 instances (cases 5 and 9). Rheumatic lesions in the heart were present in both. In 1 there was pericarditis and in the other mitral

endocarditis and myocarditis. The possibility that all the other patients also had previous independent attacks of rheumatic fever cannot, of course, be excluded, but this hardly appears likely. In all the material available for necropsy at the Mayo Clinic, the incidence of cardiac lesions of rheumatic fever is 5 per cent.

Comparison of the clinical data regarding the cardiac status of our patients with lesions subsequently demonstrated at necropsy reveals that in only 7 of the 14 cases in which rheumatic lesions occurred was there any clinical evidence of heart disease. In 6 of these (cases 3, 8, 9, 10, 12 and 13) the mitral valve was affected, and in 2 (cases 9 and 12) there was severe mitral stenosis. In 5 of the cases (cases 2, 3, 8, 9 and 10) lesions in the myocardium occurred. Subacute rheumatic pericarditis occurred in 2 (cases 2 and 3). Of the remaining 7 cases in which rheumatic heart lesions were present at necropsy, the patients in 6 were known to have had no clinical signs or symptoms of heart disease, and in 1 case there had been no clinical examination for six years before death, which occurred suddenly at the patient's home (case 6).

Of the 6 cases in which nonrheumatic lesions were observed at necropsy, signs and symptoms of heart disease were present clinically in only 2. In 1 (case 17) there was hypertrophy of the heart resulting from hypertension, and in the other (case 15) coronary thrombosis with acute and chronic infarction of the myocardium had occurred.

The causes of death in these cases of chronic infectious arthritis were varied. Cardiac disease was considered a primary or important contributory cause of death in 7 of the 14 cases in which rheumatic heart lesions were found at necropsy (cases 1, 2, 3, 6, 8, 9 and 12).

In the group of 6 cases in which nonrheumatic heart lesions were observed at necropsy, the cardiac injury was considered a contributory cause of death in 2 instances.

COMMENT

Many pathologists are of the opinion that the lesions of rheumatic fever can be diagnosed with the same degree of accuracy as can lesions of tuberculosis and syphilis. There is, however, considerable controversy regarding the specificity of the Aschoff nodule in the diagnosis of rheumatic heart disease. Fahr,[20] Rhoads,[21] Clawson[22] and others have observed similar lesions in such varying conditions as scarlet fever, meningococcic endocarditis and subacute bacterial endocarditis. Some

20. Fahr, T.: Beiträge zur Frage der Herz- und Galenkveränderungen bei Gelenkrheumatismus und Scharlach, Virchows Arch. f. path. Anat. **232**:134-159, 1921.

21. Rhoads, C. P.: Vegetative Endocarditis Due to the Meningococcus, with a Case Report, Am. J. Path. **3**:623-630 (Nov.) 1927.

22. Clawson, B. J.: The Aschoff Nodule, Arch. Path. **8**:664-685 (Oct.) 1929.

workers, notably Fahr [23] and Aschoff,[24] have denied the histologic identity of these lesions. There can be little doubt, however, that in the absence of the aforementioned diseases the typical Aschoff body should be held as evidence for rheumatic heart disease. There can also be no denial of the fact that rheumatic heart disease can often be diagnosed accurately in the absence of typical Aschoff bodies if other characteristic changes are present.

The rheumatic nature of the subacute and chronic lesions described in the first 10 cases of our series cannot be seriously disputed. Aschoff bodies in various stages of development were found in the myocardium, valves or pericardium in all but 2 cases. In these 2 (cases 9 and 10) the character of the valvular changes (thickening of the mitral leaflets, thickening and shortening of the chordae tendineae and mitral stenosis) was sufficiently characteristic to make possible an unequivocal diagnosis of rheumatic heart disease. Histologically, the diagnosis was supported by the finding of proliferation of histiocytes and fibroblasts in the valves, together with numerous perivascular scars in the myocardium.

In the 4 cases of group 3 in which histologically inactive lesions were present, our diagnosis may perhaps be questioned. The macroscopic appearance of these lesions, however, was typical of rheumatic heart disease. Thus, severe mitral stenosis occurred in 2 cases (cases 12 and 13), whereas in the other 2 there were milder, but none the less typical, changes of healed rheumatic endocarditis (thickening of mitral leaflets and chordae tendineae and in 1 instance organized verrucae on the mitral leaflet).

The histologic appearance of these lesions was characteristic of old, "burned-out" rheumatic lesions. The possibility that these changes might have been produced by some infection other than that responsible for rheumatic fever cannot of course be entirely excluded. It should be emphasized, however, that the cardiac lesions which occurred in this group differed in no way from lesions which have been repeatedly demonstrated in known cases of rheumatic fever.

Inasmuch as the argument might be advanced that some infection other than that responsible for rheumatic fever or chronic infectious arthritis was responsible for the occurrence or reactivation of the rheumatic cardiac lesions, it is interesting to note that evidence of acute terminal infection was present at necropsy in only 4 cases (bronchopneumonia in cases 4, 5 and 9 and streptococcic septicemia in case 13). Evidence of chronic infections was found in only 2 cases (pulmonary

23. Fahr, T.: Vergleichende Herzuntersuchungen bei Scharlach, Streptokokkeninfektion und rheumatischer Granulomatose, Beitr. z. path. Anat. u. z. allg. Path. **85**:445-468 (Nov.) 1930.

24. Aschoff, L.: Rheumatic Nodules in the Heart, Ann. Rheumat. Dis. **1**:161-166 (July) 1939.

tuberculosis in case 7 and chronic bronchiectasis in case 14). It is extremely unlikely that either infection was responsible for the cardiac lesions in these cases. Their possible role in the reactivation of the lesions is unknown.

We believe it is significant that in only 7 of our 14 cases in which rheumatic heart lesions were found did the patients have during life any ascertainable signs or symptoms of heart disease. This would suggest that many patients with chronic infectious arthritis may have rheumatic cardiac lesions which are not severe enough to attract the attention of either the patient or the physician. It indicates, furthermore, that one must draw a distinction between lesions which are actually present and those which are clinically apparent. This study indicates that only by careful anatomic studies can the true incidence of cardiac lesions be determined in cases of chronic infectious arthritis.

Several explanations for the comparative mildness of cardiac damage among patients with chronic infectious arthritis might be advanced. The lack of severity might be attributed to the fact that the onset of chronic infectious arthritis is, as a general rule, later than the onset of rheumatic fever. Thus, Dawson [25] stated that in 90 per cent of 257 patients with rheumatic fever the onset occurred before the age of 15 years, whereas in 90 per cent of 202 patients with chronic infectious arthritis the onset was after the age of 15 years. The average age at onset in 23 cases of the present series in which this information was available was 33.9 years. It is generally believed that the heart is more vulnerable to rheumatic infection in younger persons than in older ones.

Hench [26] has suggested that the cardiac lesions associated with chronic infectious arthritis may go unrecognized by both the patient and the physician because patients with this disease are likely to lead a relatively restricted life in which an abnormally small tolerance of exercise is not noted. The lesions might thus have greater opportunity to heal and less tendency to progress than they would among patients who have cardiac lesions and who after recovering from the articular manifestations of rheumatic fever subsequently resume full activity.

Our anatomic observations, in general, support the clinical observations that the cardiac lesions associated with chronic infectious arthritis are not as severe or as widespread as such lesions are in the hearts of young persons who have rheumatic fever. Nevertheless, the differences are of degree and not of kind. Cardiac hypertrophy (weight of the heart more than 300 Gm.) occurred in 11 of the cases in which there were rheumatic lesions, and in 7 cases of this group the cardiac injury played an important role in causing death.

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25. Dawson, M. H.: Chronic Arthritis, in Nelson's New Loose-Leaf Medicine, New York, Thomas Nelson & Sons, 1937, vol. 5, pp. 605-644.

26. Hench, P. S.: The Systemic Nature of Chronic Infectious Arthritis, Atlantic M. J. **28**:425-436, 1925.

It should also be emphasized that in all but 4 of the 14 cases there was histologic evidence of activity in the rheumatic lesions. This indicates that if a rheumatic lesion is clinically apparent, the chances are great that the process is still active. The importance of this fact should be considered in the decision regarding a patient's ability to pursue normal life and work.

The discovery in 14 of the 25 unselected cases of chronic infectious arthritis of cardiac lesions grossly and histologically similar to those associated with rheumatic fever suggests a definite relationship between these two diseases. Further evidence of this close relationship has been presented by Poynton,[27] Coates and Coombs,[28] Clawson and Wetherby,[29] Dawson [30] and Hawthorne [31] on the basis of the histologic similarity of the subcutaneous nodules of the two diseases and by Klinge [32] and his co-workers on the basis of certain anatomic similarities in the articular and the periarticular lesions, as well as in those in the heart.

The nature of this relationship is not clear and probably will not be understood until after the discovery of the etiologic agent or agents of these diseases.

SUMMARY AND CONCLUSIONS

In a study at necropsy of 25 cases of chronic infectious (rheumatoid) arthritis, cardiac lesions were demonstrated in 20. Lesions identical with those of rheumatic fever were observed in 14 (56 per cent). Nonrheumatic cardiac lesions were present in 6 (24 per cent). In 10 of the 14 cases in which there were rheumatic lesions there was histologic evidence that the inflammatory process was still active and progressive at the time death occurred. In 7 of the 14 instances associated with rheumatic cardiac lesions, the heart disease was judged to be an important factor in causing death. In only 7 of the 14 instances associated with rheumatic cardiac lesions had signs or symptoms of heart disease been present during life. The high incidence of rheumatic cardiac lesions in this series is suggestive of a relationship between chronic infectious (rheumatoid) arthritis and rheumatic fever.

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27. Poynton, cited by Hawthorne.[31]

28. Coates, V., and Coombs, C. F.: Observations on the Rheumatic Nodule, Arch. Dis. Childhood **1**:183-193, 1926.

29. Clawson, B. J., and Wetherby, M.: Subcutaneous Nodules in Chronic Arthritis: Clinical, Pathological and Bacteriological Studies, Am. J. Path. **8**:283-293 (May) 1932.

30. Dawson, M. H.: A Comparative Study of Subcutaneous Nodules in Rheumatic Fever and Rheumatoid Arthritis, J. Exper. Med. **57**:845-858 (May) 1933.

31. Hawthorne, C. O.: On Subcutaneous Nodules, Brit. J. Rheumat. **1**:109-117, 1938.

32. Klinge, F.: Der Rheumatismus; pathologisch-anatomische und experimentell-pathologische Tatsachen und ihre Auswertung für das ärztliche Rheumaproblem, Ergebn. d. allg. Path. u. path. Anat. **27**:1-351, 1933.

FAMILIAL NONHEMOLYTIC JAUNDICE

CONSTITUTIONAL HEPATIC DYSFUNCTION WITH INDIRECT VAN DEN BERGH REACTION

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Jaundice of the familial type is almost invariably considered to be hemolytic. Congenital or familial hemolytic (acholuric) icterus is a well defined disease entity. That there are instances of familial icterus in which all the evidences of excessive blood destruction are lacking has only occasionally been recognized. We have recently observed two families in which, although bilirubinemia of the "indirect" type was present, there were the signs neither of hepatic disease nor of splenomegaly, increased fragility of the red cells or reticulocytosis. The urine was free of bilirubin and did not contain increased quantities of urobilinogen, and the results of hematologic examinations were uniformly normal. Final proof of the nonhemolytic character of the icterus in these families was obtained from study of the urobilinogen content of the feces, which was either normal or slightly decreased.

From 1900 to 1907, Gilbert and his associates [1] described a series of cases of congenital or familial icterus, which they divided into three main categories: (1) physiologic cholemia (normal); (2) simple familial cholemia, and (3) simple chronic icterus. The publications of Gilbert and his collaborators have been widely cited, and the few authors who discussed the possibility of a nonhemolytic, acholuric icterus usually referred to it as "Gilbert's disease." Careful perusal of the articles by Gilbert and his associates reveals that their cases of "familial cholemia" were in all probability examples of mild hemolytic jaundice. Later, after

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1. Gilbert, A.; Lereboullet, P., and Herschen, M.: Les trois cholémies congénitales, Bull. et mém. Soc. méd. d. hôp. de Paris **24**:1203, 1907. Gilbert, A.; Castaigne, J., and Lereboullet, P.: De l'ictère familial, ibid. **17**:948, 1900.

Chauffard's extensive studies [2] of congenital hemolytic jaundice, the writings of Gilbert received diminishing attention, although his name persisted as a tag which served to identify a vague type of icterus without demonstrable pathologic change and without hemolytic character. Various authors have commented briefly on a condition which van den Bergh [3] called "physiologic hyperbilirubinemia" and which was not infrequently noted in young people. Meulengracht [4] has recently described the condition in a group of cases of mild jaundice as "icterus intermittens juvenilis." Rozendaal, Comfort and Snell,[5] in their analysis of cases of slight and latent jaundice, referred briefly to a few cases of familial nonhemolytic, nonobstructive jaundice as possible examples of "constitutional hepatic dysfunction." Tecon [6] differentiated two types of "hereditary hyperbilirubinemia:" "familial cholemia" and hemolytic icterus.

It is the purpose of this paper to present observations made on two families with chronic, usually mild, nonhemolytic, nonobstructive, acholuric retention icterus. Although superficially the disease in these families resembled mild or latent hemolytic icterus, differentiation was readily made by laboratory studies. Results of the bilirubin excretion test indicated that disturbed permeability of the hepatic cells for bilirubin (retention jaundice) was probably present. Recognition of such a condition is important so that familial hemolytic jaundice may be ruled out.

METHODS

Hemoglobin.—The Evelyn photoelectric method [7] was used. One hundred per cent equals 15.6 Gm. per hundred cubic centimeters of blood.

Red Cell Count; White Cell Count.—Pipets and hemocytometers certified by the United States Bureau of Standards and an automatic shaker were used.

Platelet Count.—The method of Dameshek [8] was employed. The normal count is 400,000 to 900,000 per cubic millimeter.

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2. Chauffard, M. A.: Pathogenie de l'ictère congénital de l'adulte, Semaine méd. **27**:25, 1907; Les ictères hémolytiques, ibid. **28**:49, 1908.

3. Hijmans van den Bergh, A. A.: Die Gallenfarbstoffe im Blute, Leiden, S. C. van Doesburgh, 1918.

4. Meulengracht, E.: Icterus intermittens juvenilis (Chronischer intermittierender juveniler Subikterus), Klin. Wchnschr. **18**:118, 1939.

5. Rozendaal, H. M.; Comfort, M. W., and Snell, A. M.: Slight and Latent Jaundice, J. A. M. A. **104**:374 (Feb. 2) 1935.

6. Tecon, R. M.: A propos d'ictères chroniques, Helvet. med. acta **5**:671 1938; Les hyperbilirubinémies héréditaires: La cholémie familiale et l'ictère hémolytique, Arch. d. mal. de l'app. digest. **28**:567, 1938.

7. Evelyn, K. A.: Stabilized Photoelectric Colorimeter with Light Filters, J. Biol. Chem. **115**:63, 1936.

8. Dameshek, W.: A Method for the Simultaneous Enumeration of Blood Platelets and Reticulocytes, Arch. Int. Med. **50**:579 (Oct.) 1932.

Reticulocyte Count.—The "dry" method, and films stained with brilliant cresyl blue and Wright's stain were used. The normal count is 0.0 to 1.0 per cent.

Hematocrit Reading.—Venous blood was collected in 5 cc. vials containing 6 mg. of dry ammonium oxalate and 4 mg. of potassium oxalate[9] and was centrifuged in Wintrobe hematocrit tubes for twenty-five minutes at 3,500 revolutions per minute.

Mean Corpuscular Volume.—This is determined by the formula

$$\frac{\text{hematocrit reading} \times 10}{\text{red blood cells, in millions}}$$

The normal range is 84 to 94 (100) cubic microns.

Mean Corpuscular Diameter.—Price-Jones curves were obtained by measuring the diameters of 200 cells directly, using a calibrated micrometer eyepiece. The average cell diameter normally is 7.4 to 7.6 microns.

Mean Corpuscular Thickness.—The indirect method used is based on the formula

$$\frac{\text{mean corpuscular volume}}{\pi \left(\frac{D}{2}\right)^3}$$

in which D equals the mean corpuscular diameter. The normal thickness is approximately 1.9 to 2.4 microns. The direct method used was the method of Dameshek (unpublished). The thickness of red cells is measured directly from rouleaux with a micrometer eyepiece; 200 cells are measured. The normal range is 1.9 to 2.2 microns.

Spherocytic Index.—The method of Heilmeyer,[10] which is based on the formula $\frac{\text{mean cell thickness}}{\text{mean cell diameter}}$, was used. The normal figure is approximately 0.25; the normal range is 0.25 to 0.33.

Biopsy of Bone Marrow.—The trephine method (Dameshek)[11] and the puncture method were used.

Fragility of Red Blood Cells to Hypotonic Solutions of Sodium Chloride.—We employed the method of Daland and Worthley,[12] in which the red cells are adjusted to constant volume after being washed three times with physiologic solution of sodium chloride.

Fragility of Red Blood Cells to Lysolecithin.—The method used was that of Singer,[13] in which lysolecithin is obtained from normal serum and tested against

9. Heller, V. G., and Paul, H.: Changes in Cell Volume Produced by Varying Concentrations of Different Anticoagulants, J. Lab. & Clin. Med. **19**: 777, 1934.

10. Heilmeyer, L.: Die Sphärocytose als Ausdruck einer pathologischen Funktion der Milz, Deutsches Arch. f. klin. Med. **179**:292, 1936.

11. Dameshek, W.: Biopsy of the Sternal Bone-Marrow, Am. J. M. Sc. **190**: 617, 1935. Dameshek, W.; Henstell, H. H., and Valentine, E. H.: The Comparative Value and Limitations of the Trephine and Puncture Methods for Biopsy of the Sternal Bone-Marrow, Ann. Int. Med. **11**:801, 1937.

12. Daland, G., and Worthley, K.: The Resistance of Red Blood Cells to Hemolysis in Hypotonic Solutions of Sodium Chloride, J. Lab. & Clin. Med. **20**: 1122, 1934.

13. Singer, K.: Das Problem der normalen und pathologischen Milzhämolyse, abstracted, Wien. Arch. f. inn. Med. **31**:161, 1937; The Lysolecithin Fragility Test, Am. J. M. Sc. **199**:466, 1940.

a constant volume of erythrocytes in geometrically increasing dilutions. A normal control and the red cells tested should give the same results. Thus far, abnormal, increased fragility has been found only in the congenital type of hemolytic icterus.

Icterus Index.—The method of Meulengracht was used. The normal range is 6 to 8; 10 equals the high limit of the normal range.

Qualitative Measurement of Bilirubin.—We employed the method of van den Bergh; "direct" and "indirect" reactions were noted.

Quantitative Measurement of Bilirubin.—We employed the method of Malloy and Evelyn,[14] using an Evelyn photoelectric colorimeter.

Urobilinogen in the Urine.—The method of Wallace and Diamond,[15] a roughly quantitative method applicable to specimens of freshly voided urine, was used. Normally a positive reaction occurs in dilutions of 1 : 10 and 1 : 20 occasionally in a dilution of 1 : 40.

Urobilinogen in the Feces.—Quantitative determination was made by the method of Watson,[16] which is based on the original method of Terwen [16a] and the modification of Fürth and Singer.[17] The normal level of urobilinogen excretion is 40 to 150 mg. per day.

Galactose Tolerance Test.—The method of Bauer [18] was used. The excretion of more than 3 Gm. signifies hepatic damage.

Bromsulphalein Test.—By the method of Rosenthal and White,[19] an injection of 5 mg. per kilogram is given. Normally, less than 15 per cent should be present in the serum after half an hour.

Bilirubin Excretion Test.—By the method of Eilbott [20a] and von Bergmann,[20b] an injection of 50 mg of bilirubin is given and the bilirubin content of the blood is measured five minutes, one hour, two hours and three hours after the injection. The normal retention is less than 15 per cent after three hours.

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14. Malloy, H. T., and Evelyn, K. A.: Oxidation Method for Bilirubin Determinations in Bile and Meconium with the Photo-Electric Colorimeter, J. Biol. Chem. **122**:597, 1938.

15. Wallace, G. B., and Diamond, J. S.: The Significance of Urobilinogen in the Urine as a Test for Liver Function, Arch. Int. Med. **35**:698 (June) 1925.

16. Watson, C. J.: The Average Daily Elimination of Urobilinogen in Health and in Disease, with Special Reference to Pernicious Anemia, Arch. Int. Med. **47**:698 (May) 1931.

16a. Terwen, A. J. L.: Ueber ein neues Verfahren zur quantitativen Urobilinbestimmung in Harn und Stuhl, Deutsches Arch. f. klin. Med. **149**:92, 1925.

17. Fürth, O., and Singer, K.: Ueber die quantitative Bestimmung kleiner Urobilinogen-und Urobilinmengen in den Faeces, Ztschr. f. d. ges. exper. Med. **69**:152, 1929.

18. Bauer, R.: Ueber alimentäre Galaktosurie, Deutsche med. Wchnschr. **34**: 1505, 1908.

19. Rosenthal, S. M., and White, E. C.: Clinical Application of the Bromsulphalein Test for Hepatic Function, J. A. M. A. **84**:1112 (April 11) 1925.

20. (*a*) Eilbott, W.: Funktionsprüfung der Leber mittels Bilirubinbelastung, Ztschr. f. klin. Med. **106**:529, 1927. (*b*) von Bergmann, G.: Zur funktionellen Pathologie der Leber insbesondere der Alkohol-Aetiologie der Cirrhose, Klin. Wchnschr. **6**:776, 1927.

Weltmann Test [21].—Serum is diluted 1:50 with solution of calcium chloride in concentrations ranging from 0.1, 0.09, 0.08 to 0.01 per cent and then is heated in a water bath at 100 C. for fifteen minutes. Normally, coagulation of the diluted serum occurs only in the first six or seven dilutions. Coagulation of the serum in the higher dilutions occurs in the presence of cirrhosis of the liver, hepatitis and occasionally various other conditions associated with hypoproteinemia.

REPORT OF CASES OF FAMILIAL NONHEMOLYTIC JAUNDICE

Ro. Family.—1. Arthur, aged 20, of Jewish origin, consulted one of us (W. D.) in March 1939 because of icterus.

The patient's paternal grandfather had been known to have jaundice for many years and had been studied for this condition at several hospitals. He died at the age of 64. The patient's father, who died of pneumonia at the age of 28, had also been known to have mild jaundice for several years; no records of the type of jaundice are available. The patient's mother was healthy. An only brother was said to be free of jaundice.

The mother stated that the patient was not jaundiced at birth. Jaundice had first been noted at the age of 4, directly after an operation for appendicitis. Since then jaundice had been constantly present. Subjective complaints were few, although the patient frequently felt tired and without "pep." He seemed unable to gain weight. The jaundice was variable in intensity and was perhaps somewhat increased by ingestion of fried and fatty foods.

Examination revealed a thin boy with moderate icterus of the scleras and the skin. Hepatic dulness began in the fourth right interspace and extended to the costal margin; the edge of the liver was not felt. Splenic dulness was not increased, and the edge was not palpable on deep inspiration. The remainder of the examination showed nothing abnormal.

The urine showed no bilirubin, and the urobilinogen content (Wallace-Diamond technic) was 1:20 on three occasions and 1:40 on three others. The stools were brown but definitely low in their content of urobilinogen. The icterus index was elevated (35 to 60 units), and there was a well marked increase in bilirubin, which gave the "indirect" van den Bergh reaction. The total cholesterol of the blood measured 145 mg. per hundred cubic centimeters and the cholesterol esters 101 mg. The blood proteins were normal in amount.

The blood was essentially normal. There was a slight increase in hemoglobin and in the red cell count. The reticulocyte count in seven examinations was 0.0, 0.0, 0.5, 0.2, 0.2, 0.0 and 0.0 per cent. The fragility of the red cells to hypotonic solutions of sodium chloride was 0.46 to 0.24 per cent. Their fragility to lysolecithin was normal. The average diameter of the red cells was 6.9 microns, the mean cell volume 88.9 cubic microns and the mean cell thickness 2.3 microns. Spherocytosis was completely absent, and the red cells, in all fresh preparations, including those showing rouleaux, were of normal thickness.

A sternal puncture revealed a normal cellular marrow. The ratio of nucleated red cells to granulocytes was 0.53 to 1. There was no evidence of erythroblastic hyperplasia or immaturity. The differential count of 500 marrow cells showed:

21. Weltmann, O.: Ueber die Spiegelung exsudativ-entzündlicher und fibröser Vorgänge im Blutserum, Med. Klin. **1**:240, 1930. Rosegger, H.: Das Weltmann'sche Koagulationsband, Ergebn. d. inn. Med. u. Kinderh. **57**:183, 1939.

erythrogones 0.4 per cent; normoblasts A 1.8 per cent, normoblasts B 11.2 per cent, normoblasts C 22.4 per cent; myeloblasts 2.6 per cent; myelocytes 10.2 per cent; metamyelocytes 22.6 per cent; mature polymorphonuclear leukocytes 17.6 per cent; eosinophils 8.4 per cent; basophils 0.2 per cent, and histiocytes 1.6 per cent.

The liver function tests gave negative results: The galactose tolerance test showed a total excretion of 2.3 Gm. in four hours. The bromsulphalein test showed 4 per cent retention of dye after thirty minutes. Gastric analysis revealed a maximum concentration of 32 units of free hydrochloric acid. The concentration of bilirubin in the duodenal drainage was 14.7 mg. per hundred cubic centimeters (normal); no cholesterol or other abnormal crystals were seen. Roentgenograms of the gallbladder and of the gastrointestinal tract were entirely normal.

Continued observation of this patient has revealed that he has constant, although somewhat intermittent, icterus. No change in icterus occurred with a fat-free diet or after the administration of sodium dehydrocholate by mouth.

2. Jerome, aged 18 (only sibling of Arthur), was completely asymptomatic. On examination, questionable icterus of the scleras was observed. The icterus index was 15, and quantitative determination of bilirubin gave 1.61 mg. per hundred cubic centimeters. The remainder of the laboratory data are summarized in table 1.

Ru. Family.—Nancy (L.) was the first member of the family to be observed. In 1936 she was said to have mild congenital hemolytic icterus. At that time studies were also made of two sisters, Susie and Josephine, but because our interest in hemolytic icterus was then only slight, no extensive investigations were carried out. In 1939 all the members of the family were recalled and systematic studies were undertaken.

The Ru. family was of Italian origin, the father and mother having come to the United States in 1910. The family consisted of the parents and 8 children, 3 boys and 5 girls.

1. Lawrence Sr., the father, aged 59, was a farmer. He remembered that his mother was "yellow" and that her eyes were always "yellow." He was not certain about yellow coloration in any of his brothers or sisters. A first cousin, the son of Mario S., a maternal uncle (see page 270), was always yellow, as was the cousin's son. When Lawrence Sr. was 18 it was remarked that his face had a peculiar yellow color. At 30 he was said to have "liver trouble," his face was "all green" for "about a year" and he had abdominal pain. He believed that since then his color had been normal. For about seven or eight years he had known that he had hypertension. In the fall of 1937 transient left hemiplegia had occurred. At the time of this writing he felt well. Examination revealed a healthy-appearing, well developed and well nourished man with definite icterus of the scleras and the skin. Hepatic dulness was noted from the fourth right interspace to the costal margin; the edge of the liver was not felt. Splenic dulness was not increased; the edge of the spleen was not palpable. The remainder of the examination revealed nothing abnormal except for slight hypertension with a pressure of 174 systolic and 90 diastolic. There was no modification of the icterus by the administration of tablets of dehydrocholic acid.

The laboratory data are summarized in table 1. The results of the bilirubin excretion test indicated a grossly pathologic condition, 64 per cent retention being observed at the end of three hours (fig. 1).

2. John, aged 28, was born in the United States. He had had slight attacks of nausea and vomiting every five or six months, usually after a drinking bout; otherwise he was healthy. Definite icterus of the scleras and questionable icterus

TABLE 1.—*Laboratory Data in Cases of Nonhemolytic and Hemolytic Jaundice*

Name	Hemoglobin (Evelyn) Per Cent	Hemoglobin (Evelyn) Gm.	Red Blood Cells	White Blood Cells	Platelets	Reticulocytes, per Cent	Hematocrit Readings, per Cent	Mean Corpuscular Volume, Cubic Microns	Mean Corpuscular Diameter, Microns	Mean Corpuscular Thickness, Microns	Spherocytic Index (Normal, 0.25 to 0.34)	Fragility: Sodium Chloride	Fragility: Lysolecithin	Blood Bilirubin: Icterus Index	Blood Bilirubin: Quantitative, Mg. per 100 Cc.	Fecal Urobilinogen, Mg.
I. Familial nonhemolytic jaundice																
Ro.																
Arthur.....	109	17.0	5,830,000	10,000	573,800	0.2	52.0	88.9	6.9	2.3	0.33	0.46 to 0.24	Normal	35.0 to 63.0	5.2 to 13.1	18.0
Jerome.....	104	16.2	5,850,000	11,300	514,800	0.7	50.0	85.4	7.0	2.2	0.31	.....	.....	15.0	1.61	....
II. Familial nonhemolytic jaundice																
Ru.																
Lawrence Sr...	99	15.3	4,600,000	8,100	.....	0.2	47.0	102.0	7.0	2.6	0.37	0.44 to 0.20	Normal	18.0	2.14	63.0
Lawrence Jr...	93	14.5	5,060,000	4,800	.....	0.2	44.0	86.9	6.8	2.3	0.34	0.42 to 0.24	Normal	20.0	1.87	16.0
John.....	100	15.6	5,270,000	7,000	.....	0.0	46.0	87.2	7.0	2.2	0.31	0.42 to 0.24	Normal	15.0	1.41	....
Tony.....	95	14.8	4,530,000	4,800	.....	0.0	46.5	102.0	6.9	2.7	0.39	0.44 to 0.24	Normal	18.0	1.41	58.5
Josephine.....	93	14.4	4,660,000	7,900	366,100	0.2	41.0	87.9	7.1	2.2	0.31	0.44 to 0.30	Normal	20.0	3.04	53.0
Susie.....	84	13.1	4,020,000	6,100	313,500	0.4	38.0	94.5	7.3	2.2	0.30	0.48 to 0.24	Normal	15.0 to 25.0	2.45	50.0
Mary.....	80	12.5	4,000,000	6,000	776,000	1.3	36.5	91.0	7.1	2.2	0.31	0.42 to 0.24	Normal	20.0	2.90	34.0
Nancy (L.)....	89	13.8	4,350,000	8,800	504,000	1.1	39.5	90.8	7.1	2.3	0.33	0.44 to 0.24	Normal	17.5	1.83	44.0
Caroline.....	84	13.1	4,530,000	9,800	.....	0.4	40.0	88.3	7.6	1.97	0.26	0.42 to 0.24	Normal	6.0	0.79	34.0
III. Familial hemolytic jaundice																
Lawrence N...	112	17.5	5,360,000	7,800	900,000	0.5 to 4.6	46.0	85.5	6.8 to 7.1	2.4 to 2.6	0.31	0.56 to 0.16	Increased	35.0	3.2 to 5.4	440.0
Robert B.....	97	15.1	4,720,000	8,100	405,000	1.3 to 5.0	42.0	87.9	6.9	2.3	0.33	0.52 to 0.20	Increased	11.0	0.7	430.0

of the skin were observed. Hepatic dulness was somewhat increased and the edge of the liver was just palpable. The spleen was not felt.

3. Tony, aged 26, was born in the United States. He had had pneumonia in childhood and scarlet fever at the age of 10, but had no complaints at the time of study. He was a healthy-appearing man with slight, though distinct, icterus of the scleras and the skin. There was no enlargement of the liver or the spleen.

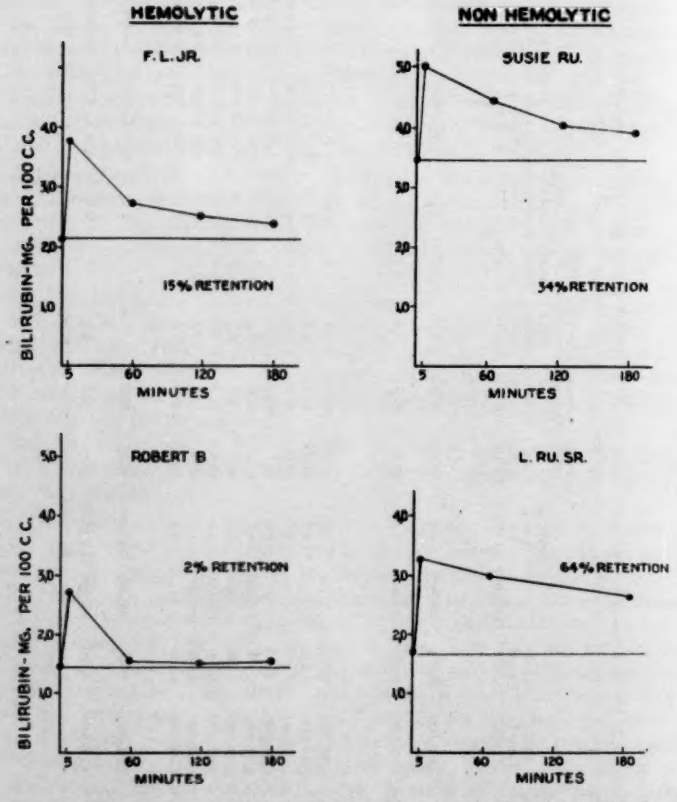

Fig. 1.—The results of the bilirubin excretion test in 2 patients with familial hemolytic icterus as compared with results in 2 patients with the nonhemolytic variety. Note that the bilirubin retention in the former patients is normal, although approximately the same degree of bilirubinemia is present in the two groups of patients.

4. Lawrence Jr., aged 16, was born in the United States. His past history was normal, and he had no complaints at the time of study. He was a healthy-appearing boy with definite icterus of the skin and scleras. Enlargement of the liver or the spleen was not detected by percussion or palpation.

In general, the male members of the family had not noticed icterus (except for the father, years previously) and felt well. They were uncooperative in the investigations. The female members had apparently paid more attention to themselves, knew of their yellowish coloration and usually complained of fatigue, lack of energy and occasional headaches. They were far more amenable to investigative studies.

5. Nancy, aged 27, complained of fatigue and exhaustion in May 1936. At first she stated that for about ten years occasional changes in the color of her face had been noted by her companions; at a later visit she stated that she had found that her jaundice had been noted since birth and had been intermittent. After the birth of her second child (by cesarean section), in 1934, she had failed to regain her normal vigor and complained of fatigue, dizziness, epigastric pain, headache and attacks of trembling, at times relieved by vomiting. After an attack, which might have had some relation to the menstrual cycle, she noticed that her skin became more yellow. Her appetite was poor, and various foods, such as pork, oranges and strawberries, caused gastric distress. Examination in May 1936 showed definite jaundice of the skin and the scleras. All further examinations revealed nothing abnormal, except for the icterus.

The laboratory data for Nancy are summarized in table 1. The icterus index varied from 18 to 23; the bilirubin content of the blood was approximately 2 mg. per hundred cubic centimeters. The fragility test gave a value of 0.42 to 0.32 per cent in June 1936, 0.42 to 0.34 per cent in October 1936 (drop method) and 0.44 to 0.24 per cent in 1939 (method of Daland and Worthley). In 1936 the hemoglobin varied from 82 to 85 per cent (Sahli), the red cell count from 4,000,000 to 4,880,000 and the white cell count from 7,300 to 9,300. The reticulocytes were 2 per cent. Biopsy of the bone marrow of the sternum (trephine method) yielded essentially normal findings, with no evidence of erythroblastic hyperplasia. The urine was normal. The cholesterol content of the blood was 202 mg. per hundred cubic centimeters. The Hinton and Kahn reactions were negative. Roentgenograms of the gallbladder were normal. A series of roentgenograms of the gastrointestinal tract showed nothing abnormal except an inconstant pressure defect on the superior surface of the duodenal cap. Special hematologic studies in 1939 (when the patient was three months pregnant) showed normal values for hemoglobin and normal erythrocyte, leukocyte and thrombocyte counts. The reticulocyte value was 1.1 per cent. The average diameter of the red cells was 7.1 microns, with a variation in size from 5.3 to 9.0 microns. The mean corpuscular volume was 90.8 cubic microns, the mean cell thickness 2.3 microns and the spherocytic index 0.33. The resistance to hypotonic solutions of sodium chloride was 0.44 to 0.24 per cent; the fragility to lysolecithin was normal; the blood bilirubin gave an indirect reaction; there was no increase in the urinary urobilinogen, and the urobilinogen content of the feces was 44 mg. per day (normal value, 40 to 150 mg.).

6. Susie, aged 20, in October 1936 complained of headaches, dizziness, fatigue and constipation. At another time she stated that since the age of 15 she had noted a yellowish coloration of the skin and eyes, which she believed became aggravated with excitement, nervousness, fatigue and eating fried and fatty food and chocolate. A physical examination showed nothing abnormal except for perceptible icterus of both the skin and the scleras.

The patient was restudied in 1939. The laboratory data are summarized in table 1. The values for hemoglobin and the erythrocyte, leukocyte and platelet counts were essentially normal in April 1939, although in July and August 1939 there was evidence of slight normocytic anemia (hemoglobin 75 per cent, red cell

count 3,780,000). The reticulocyte count was 0.2, 0.2 and 0.4 per cent. The mean diameter of the red blood cells was 7.3 microns, the mean cell volume 94.5 cubic microns, the mean cell thickness 2.2 microns and the spherocytic index 0.30. The thickness measured directly from rouleaux was 2.1 microns, and the photomicrographs of rouleaux showed normal cells, with no tendency toward increased thickness. The fragility of the red cells to hypotonic solutions of sodium chloride was 0.48 to 0.24 per cent; the fragility to lysolecithin was normal. The urine showed no increase in urobilinogen; the icterus index of the blood varied from 15 to 25 units and the bilirubin content of the blood was 2.45 mg. per hundred cubic centimeters. The urobilinogen content of the feces was 50 mg. per day. The bromsulphalein test for hepatic function showed a 2 per cent retention after thirty minutes (normal).

7. Josephine, aged 23, had always considered herself well except for frequent fatigue and lack of "pep," together with noticeable sallowness. Examination disclosed slight icterus of the scleras and a sallow appearance of the skin. Neither the liver nor the spleen was palpable. The laboratory data are recorded in table 1.

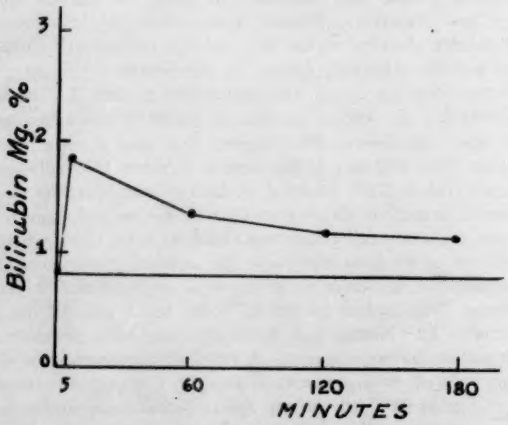

Fig. 2.—Result of the bilirubin excretion test in Caroline Ru., the only one of the Ru. children without definite bilirubinemia. At the end of three hours there is 33 per cent retention of injected bilirubin (normal retention up to 15 per cent), despite the absence of icterus.

8. Mary, aged 19, also felt tired from time to time. Examination revealed nothing abnormal except for slight icterus of the scleras and the skin. The laboratory data are recorded in table 1. The result of the bromsulphalein excretion test was normal.

9. Caroline, aged 25, felt well except for slight asthenia. On examination she seemed somewhat more vigorous and robust than her sisters. There was no icterus of the scleras, and the skin did not present the sallow, yellowish appearance of her sisters. Although the bilirubin content of the serum was within normal limits (0.79 mg. per hundred cubic centimeters), the response to the bilirubin excretion test was definitely, though slightly, pathologic, since only 66 per cent of the injected bilirubin was excreted after three hours (normally 85 per cent is excreted).

ANALYSIS OF CASES, INCLUDING THOSE IN THE LITERATURE

Onset of Disease.—This cannot ordinarily be ascertained, although patients or their relatives will occasionally date the onset of the jaundice by some specific event, such as an operation (Arthur Ro.) or an illness (Lawrence Ru. Sr.). Rozendaal and his associates reported several cases in which jaundice had been observed at birth and had been constantly present until adult life.

Familial Character.—This has been stressed by Tecon, who stated that one must discriminate between two types of hereditary hyperbilirubinemia: "familial cholemia" and hemolytic icterus. He reported on 2 families, family Cr., 2 members, and family G., 3 members. It should be stated that the record of family Cr. is not convincing, since the only abnormality observed in "Maurice" of this family was a bilirubin content of the blood of 0.85 mg. per hundred cubic centimeters; his brother Pierre's bilirubin content varied from 1.06 to 1.5 mg. The early descriptions of Gilbert and his associates dealing with "familial cholemia" were in all probability, as stated previously, those of mild congenital hemolytic icterus. Van den Bergh, in his description of "physiologic hyperbilirubinemia," stated that he had observed a case of familial occurrence. Rozendaal, Comfort and Snell, in analyzing 214 cases of mild icterus, found 34 instances in which the disease could be classified as "constitutional hepatic dysfunction." From the patients in several of these cases a familial history of icterus was obtained, although the other members of the family were not examined. In 1 case which was cited, a man aged 32 had "always" been slightly jaundiced; his mother, 5 brothers and 1 sister were similarly affected, although otherwise in good health (hematologic and chemical details were not given). In Meulengracht's series of 24 cases, icterus was present in 3 members of 1 family, and either a brother or a sister of the patients in 2 other cases was also said to have icterus. (Laboratory data were not given.)

The method of transmission in these cases can only be speculated on, since so few cases have been described. In both our families transmission was through the father. However, the elder Ru.'s mother was probably the transmitter, and his sister had apparently transmitted the condition to her son. The mother was also apparently the transmitter in 1 of the cases of Rozendaal and his associates. It would seem, therefore, that both parents may transmit the disease directly to their children. Of the 8 children in the Ru. family, all were affected (Caroline only slightly); this might indicate that the disorder was transmitted as a mendelian dominant character.

Age, Sex, Social Status and Race.—Meulengracht stressed the juvenile character of the condition ("icterus intermittens juvenilis") and stated that in time it might disappear. In 1 of the cases reported

by Rozendaal and his collaborators, the mother of a patient aged 32 had shown continued icterus to the time of the report. Although the great majority of our patients were between 20 and 25 years of age, 1, Lawrence Ru. Sr., was 60. The latter's first cousin, aged 51, was also definitely icteric. In the Ro. family, the father of the 2 boys studied had been known to have jaundice, while the grandfather had been jaundiced for many years at the time of his death, at the age of 64.

Both sexes appear to be similarly affected. Although Meulengracht observed the condition most commonly in members of the medical profession, the apparent frequency of occurrence, as he pointed out, might have been due to the more careful observation of slight abnormalities by members of this group. Our own cases occurred in families without medical associations. With reference to racial occurrence, the disorder has been described from France,[1] Switzerland,[6] Holland,[3] Denmark [4] and this country.[5] In our own cases, the Ru. family was of Italian origin and the Ro. family was Jewish.

Symptoms.—Meulengracht [4] stated that the histories of all patients with this type of jaundice showed an "extraordinary monotony" characterized by fatigue and asthenia. The periods of fatigue were usually consistent with periods of increased jaundice. In our own cases, the same "monotony" of symptoms was present in both the Ro. and the Ru. family, although not as a constant feature. In the Ro. family, 1 member, who was perceptibly jaundiced, frequently felt tired and without "pep"; the other, who was subicteric, had no symptoms. In the Ru. family, the symptoms of frequent fatigue, gastric distress and asthenia were limited to the female members. It is possible that these symptoms were chiefly neurasthenic in origin and completely unrelated to the icterus. The male members of the family, although having the same degree of icterus, were completely asymptomatic and able to do heavy manual work.

Physical Signs.—Except for icterus, the results of physical examination were essentially normal. The icterus varied in intensity from patient to patient and in the same person from time to time. In most of the members of the Ru. family the icterus was discernible only when the scleras were exposed (subicterus); in Susie, and at times in Nancy, the jaundice was, however, apparent at a glance. This was the case in Arthur Ro.; the latter's brother, Jerome, however, showed only icterus of the scleras. It is apparent that most cases are, in all likelihood, missed because of the slight degree of icterus present; many persons are thought merely to have a "sallow" complexion or "anemia." Our own patients were, for the most part, considered a little sallow or pallid but otherwise normal. The edge of the liver was occasionally felt. We

were unable to feel the edge of the spleen in any of our patients, nor was the splenic dulness increased to percussion. No other abnormal physical findings were discovered.

Laboratory Data.—The most important laboratory finding, and of course a prerequisite in the diagnosis, was the bilirubinemia. This was usually moderate, the amount of bilirubin ranging in the Ru. family from 1.41 to 3.07 mg. per hundred cubic centimeters with an associated icterus index of 15 to 25. In the case of Arthur Ro. the bilirubin level was distinctly above the subicterus level and ranged from 5.2 to 13.1 mg. per hundred cubic centimeters, with an associated icterus index of 35 to 60 units. However, this patient's brother had icterus indexes corresponding to those in our least jaundiced patients: icterus index 15, bilirubin level 1.61 mg. per hundred cubic centimeters. Most of the patients in Meulengracht's 24 cases had a slight degree of icterus, 13 of them presenting an icterus index between 10 and 15 units. Only 3 had icterus indexes between 20 and 25 units. In the cases reported by Tecon the icterus was also extremely mild, with the bilirubin levels ranging from 0.85 to 1.6 mg. per hundred cubic centimeters. Of the cases cited by Rozendaal and his co-workers, the bilirubin level in 1 varied from 2.8 to 3.4 mg. and in another from 3.4 to 4.2 mg. per hundred cubic centimeters. The bilirubin was always of the "indirect" variety.

Examination of the urine was uniformly negative for bilirubin and for increased amounts of urobilinogen.

Blood: In our cases the hematologic findings were essentially normal as regards the values for hemoglobin and the erythrocyte, leukocyte, thrombocyte and reticulocyte counts. For the Ro. boys the hemoglobin content, the erythrocyte counts and the hematocrit levels were at high normal values. In the Ru. family the women had red blood cell counts which were at low normal values—from 3,670,000 to 4,660,000—and the hemoglobin concentrations ranged from 76 to 93 per cent. The Ru. men also had low normal red cell counts (for men), ranging from 4,530,000 to 5,270,000. Despite the slight tendency to anemia in this family, the hemoglobin values were for the most part normal; this normality resulted in a color index just above 1 in several instances. The hematocrit–red cell relationship (mean corpuscular volume) was within the normal range, 85.4 to 94.5 cubic microns in all cases except 2, in which there was a possible increase (102 cubic microns). The only possible hematologic abnormality which could be ascertained in our cases was a distinct tendency to microcytosis, since the diameter of the red cells ranged from 6.8 to 7.3 microns in dry stained films. This observation was repeatedly checked. The coexistence of erythrocytes somewhat smaller than normal and a normal mean corpuscular volume

should indicate a mean corpuscular thickness which is either at the upper limit of normal or just above it. However, careful measurements of thickness made directly from rouleaux in these cases, together with the study of rouleaux from photomicrographs, failed to show any evidence of increased thickness. In fact, the red cells in photomicrographs of rouleaux seemed at times somewhat thinner than normal. The possible inaccuracy of the measurements for "mean corpuscular thickness" and of the "spherocytic index" as derived from the red cell count, the hematocrit readings and the mean cell diameter has been commented on by Price-Jones, Vaughan and Goddard,[22] Ponder[23] and Barrett.[24] These measurements are based on the postulate that the red cell is a short cylinder, whereas it is actually a biconcave disk. The earlier investigators presented as normal limits for thickness 1.729 to 2.545 microns. The measurements of thickness in our cases fall well within this range. The association of definite, though slight, microcytosis with a normal cell volume in our cases is difficult to explain. In view of our observations made directly on fresh preparations of blood, we are, however, inclined to disregard borderline figures obtained for the mean corpuscular thickness by the indirect method.

The resistance of the red cells to hypotonic solutions of sodium chloride was normal in every patient tested; since this test may be considered an indirect measurement of red cell thickness, the normal findings tend to confirm the absence of increased thickness. In cases of congenital hemolytic icterus with similar values for corpuscular thickness (table 1) the result of the fragility test is definitely abnormal. No evidences of increased regenerative activity on the part of the marrow were present in any case, since the leukocyte, granulocyte, platelet and reticulocyte counts always were well within the normal limits.

The hematologic findings in the few cases reported in the literature were within normal limits, although complete reports were not usually given. Meulengracht stated that the mean diameter of the red cells in his cases was normal, varying between 7.8 and 8.0 microns as determined by measurement of the fresh red cells in their own plasma. However, Gripwall,[25] in a recent study using the same method, stated that normal values range from 8.2 to 8.6 microns, with an average value

22. Price-Jones, C.; Vaughan, J. M., and Goddard, H. M.: Hematological Standards of Healthy Persons, J. Path. & Bact. **40**:503, 1935.

23. Ponder, E.: The Mammalian Red Cell and the Properties of Haemolytic Systems, in Chambers, R., and others: Protoplasma-Monographien, Berlin, Verlagsbuchhandlung Gebrüder Borntraeger, 1934, vol. 6.

24. Barrett, A. M.: A Special Form of Erythrocyte Possessing Increased Resistance to Hypotonic Saline, J. Path. & Bact. **46**:603, 1938.

25. Gripwall, E.: Zur Klinik und Pathologie des hereditären hämolytischen Ikterus, Acta med. Scandinav., 1938, supp. 96, p. 1.

of 8.4 microns. This might indicate that the cells in Meulengracht's cases, as in our own, were somewhat smaller than normal.

Bone Marrow: Specimens for biopsy were taken in 2 of our cases, and the observations were all within normal limits. There was no evidence of erythroblastic hyperplasia, and the relationship of the nucleated red cells to the white cells was normal.

Stools: Most accounts of the disorder have failed to mention the appearance of the stools. The important matter of the urobilinogen excretion has, furthermore, never been studied. In our cases the stools were always of a normal brown color. The daily excretion of urobilinogen was always either normal or somewhat diminished.

Liver Function Tests.—When performed, the galactose tolerance and bromsulphalein tests yielded normal results. The Weltmann reaction was also normal (coagulation up to seven dilutions) in all the patients tested. The reaction to injected bilirubin was abnormal; excretion was greatly delayed, and the resultant retention of bilirubin three hours after injection was thus much greater than the high normal value of 15 per cent. Of great interest was the presence of a slightly abnormal curve in 1 of the patients (Caroline Ru.), indicating a possible latent tendency toward bilirubinemia, although this condition was not present.

Prognosis.—From the standpoint of the person's general health the condition appears to have but little significance. Chauffard's remark concerning patients with familial hemolytic icterus seems even more apt for patients with the nonhemolytic type: "The patient is more icteric than sick." That the father of the Ru. family presented no greater degree of icterus than his children points to the benign, nonprogressive character of the disorder. It is possible, however, that examples of more severe forms of the disease will be found and that in these the evidences of progressive hepatitis will be present.

Treatment.—Treatment with low fat diets and with choleretics (sodium dehydrocholate) in 2 cases failed to make any impression on the jaundice. More drastic forms of treatment of such a mild disorder did not appear to be indicated.

COMMENT

There are two aspects of this problem which are worthy of comment: (1) Can one be certain that the condition described is a well defined syndrome, clearly to be differentiated from familial hemolytic icterus and chronic mild hepatic disease? (2) Assuming the existence of such a disease entity, what are the possible pathologic mechanisms involved?

Is Familial Nonhemolytic Icterus a Well Defined Syndrome?—Familial icterus is ordinarily assumed to be hemolytic. That a nonhemolytic variety exists is usually not recognized. Numerous studies on

cases of both types of familial icterus have led us to conclude that they can be clearly differentiated.

A. Differentiation from Familial Hemolytic Icterus: In the presence of chronic slight icterus in which the blood bilirubin is of the "indirect" variety, the diagnosis of mild or latent hemolytic icterus is

TABLE 2.—*Comparison of Two Patients with Hemolytic Icterus and Nonhemolytic Icterus and Approximately the Same Level of Bilirubin, Percentage of Hemoglobin and Number of Red Cells*

	Lawrence N. (Hemolytic Icterus), Aged 28	Arthur R. (Familial Nonhemolytic Icterus), Aged 21
Family history	Nothing abnormal	Grandfather, jaundice; father, jaundice; brother, slight jaundice
Past history	Rheumatic fever; rheumatic heart disease	Appendectomy at 3
Present illness	Anorexia; fatigue; jaundice noticed by friends	Weight below normal; no symptoms; jaundice noticed by friends
Physical examination	Icterus; †spleen palpable 3 finger-breadths	Icterus; spleen not felt
Blood picture	Hemoglobin, 110 per cent	Hemoglobin, 109 per cent
	Red blood cells, 5,700,000	Red blood cells, 5,730,000
	White blood cells, 8,200	White blood cells, 10,000
	Platelets, 610,000	Platelets, 573,000
	†Reticulocytes, 0.5, 2.7, 4.6, 0.8, 1.2 %	Reticulocytes, 0.0, 0.0, 0.5, 0.2, 0.2, 0.0, 0.0 %
	Hematocrit reading, 45, 46, 48, 46%	Hematocrit reading, 52, 56, 54%
	Mean corpuscular volume, 83 to 94 cubic microns	Mean corpuscular volume, 91 to 98 cubic microns
	Mean corpuscular diameter, 6.8 to 7.1 microns	Mean corpuscular diameter, 6.9 to 7.0 microns
	Mean corpuscular thickness, 2.1 to 2.6 microns	Mean corpuscular thickness, 2.3 to 2.5 microns
	†Fragility of the red blood cells to hypotonic solutions of sodium chloride, 0.72 to 0.04, 0.50 to 0.16, 0.48 to 0.16, 0.56 to 0.16 per cent	Fragility of the red blood cells to hypotonic solutions of sodium chloride, 0.46 to 0.24, 0.44 to 0.24, 0.46 to 0.30, 0.42 to 0.20 per cent
	†Fragility of the red blood cells to lysolecithin positive	Fragility of the red blood cells to lysolecithin normal
	†Rouleaux, thick cells	Rouleaux normal
	†Stained smear, spherocytosis	Stained smear, no spherocytes
	†Wet isotonic preparations, "cups" common	Wet isotonic preparations, no "cups"
Other laboratory data	Icterus index, 35, 40, 38 units	Icterus index, 35, 50, 50, 60, 45 units
	Bilirubin, 5.4, 3.2 mg. per 100 cc.	Bilirubin, 10.8, 5.2, 9.6, 13.1 mg. per 100 cc.
	Van den Bergh reaction indirect	Van den Bergh reaction indirect
	Urine, bile absent	Urine, bile absent
	Urobilinogen, 1:40, 1:80, 1:20	Urobilinogen, 1:40, 1:20, 1:20, 1:20
	†Stool, urobilinogen, 440.0 mg.	Stool, urobilinogen, 18 mg.
	†Bone marrow, hyperplasia of red blood cells	Bone marrow, normal

† An important differential point.

usually made even in the absence of a positive family history, splenomegaly, anemia, spherocytosis, an increased fragility of the red cells to hypotonic solutions of sodium chloride, reticulocytosis and an increased output of urobilinogen in the urine. Gänsslen [26] described cases of the "latent" form in which many of these symptoms were

26. Gänsslen, M.: Die hämolytische Konstitution, Klin. Fortbild. **4**:607, 1936; Der hämolytische Ikterus; hämolytische Konstitution, Klin. Wchnschr. **6**:929, 1927.

absent; for example, in 10 per cent of his cases the red blood cells showed a normal fragility to hypotonic solutions of sodium chloride, and in 30 per cent the spleen could not be palpated. That cases of very

TABLE 3.—*Differentiation of Familial Hemolytic, Familial Nonhemolytic and Hepatic Jaundice*

Signs and Symptoms	Nonhemolytic Jaundice	Latent Hemolytic Jaundice	Manifest Hemolytic Jaundice	Slight "Liver Damage," Slight Parachymatous Jaundice	Fully Developed Hepatic Disease and Hepatic Jaundice
Familial history....	Present	Present	Present	Absent	Absent
Jaundice.....	Slight to moderate	Absent or slight	Moderate	Slight	Distinct
Size of liver (palpation)	Normal	Normal	May be enlarged	May be normal	May be enlarged
Size of spleen (palpation)	Normal	Usually palpable	Readily palpable	May not be enlarged	May be readily palpable
Bilirubin in urine...	Absent	Absent	Absent	Usually absent except in serial examinations	Present
Urobilinogen in urine	Not increased	Usually increased; may be normal	Increased	Usually increased	May be absent (obstruction)
Anemia.....	Absent	Absent	Present	Absent	Frequently present
Spheroeytosis of red cells	Absent	Present	Present	Absent	Absent
Fragility of red cells	Normal	May be only slightly increased	Increased	Normal	May be decreased
Reticulocytosis.....	Absent	May be absent on single examination; always present on serial examinations	Present	Absent	Absent
Hyperplasia of bone marrow	Absent	Present	Present	Absent	May be present if anemia is present
Liver function tests	Results negative except for impaired bilirubin excretion	Results negative	Negative	May be negative	Positive (pathologic)
Feces:					
Color.....	Normal	Dark	Dark	Normal	Hypocholic
Urobilinogen content	Normal or diminished	Increased	Increased	Normal	Diminished
Bilirubin in blood..	Increased; indirect van den Bergh reaction	Increased; indirect van den Bergh reaction	Increased; indirect van den Bergh reaction	Increased; may show indirect van den Bergh reaction	Increased; direct van den Bergh reaction

mild congenital hemolytic icterus exist cannot be denied. However, even in these, one or several of the aforementioned symptoms are observed during the course of several examinations.

The following 2 cases of mild hemolytic icterus are illustrative.

Robert B., a medical student aged 24, had known of the presence of slight jaundice for many years. His father's father had been jaundiced, and his father had been jaundiced for many years. Of 3 siblings, a brother and sister were jaundiced.

Crises had never occurred in any member of the family, and none had required splenectomy. He was a rather thin, otherwise healthy-appearing young man with slight icterus of the scleras. The edge of the spleen was just felt at the costal margin. Laboratory data were as follows: hemoglobin concentration 97 per cent; red cell count 4,760,000 and white cell count 8,100; platelet count 405,000; reticulocyte count 1.3, 3.7, 4.0, 1.7, 1.9, 2.6, 4.4 and 3.9 per cent at successive observations; hematocrit reading 42 per cent; mean corpuscular volume 88 cubic microns; mean corpuscular diameter 6.9 microns; mean corpuscular thickness (indirect method) 2.3 microns and mean corpuscular thickness (direct method) 2.4 microns. Preparations of rouleaux showed definite increase in thickness with well defined individual variation. Fragility of the red cells to hypotonic solutions of sodium chloride was 0.52 to 0.20 per cent; fragility to lysolecithin was increased. No bilirubin or increased urobilinogen excretion occurred in the urine. The icterus index of the blood was 11 units, and the bilirubin content was 0.8 mg. per hundred cubic centimeters. The daily urobilinogen excretion in the feces was 430 mg. The result of the bilirubin excretion test was normal, since 98 per cent of the injected dye was excreted in three hours.

Lawrence N., a 28 year old truck driver with an entirely normal family history, had rheumatic fever and rheumatic heart disease in 1932. While he was in the hospital at that time jaundice and splenomegaly were noted, and they have been present since. He was thin and definitely icteric. Examination of the heart revealed stenosis of the mitral valve. The edge of the spleen was felt 3 fingerbreadths below the left costal margin. Laboratory data were as follows: hemoglobin concentration 110 to 116 per cent (Evelyn); red cell count 4,810,000 to 5,700,000 and white cell count 4,900 to 8,200; platelet count 610,000 to 904,000; reticulocyte count 0.5, 2.7, 4.6, 0.8 and 1.2 per cent at successive observations; hematocrit reading, 45 to 48 per cent; mean corpuscular volume 83 to 94 cubic microns; mean corpuscular diameter 6.8 to 7.1 microns; mean corpuscular thickness 2.35 to 2.60 microns. The rouleaux were increased in thickness; stained smears showed typical spherocytes; the fragility to hypotonic solutions of sodium chloride was 0.72 to 0.04, 0.50 to 0.16, 0.48 to 0.16 and 0.56 to 0.16 per cent on various tests; fragility to lysolecithin was increased. The urine showed no bilirubin; urobilinogen concentration in the urine varied from 1:2 to 1:80. The icterus index of the blood varied from 35 to 40 units; the bilirubin content (indirect method) varied from 3.2 to 5.4 mg. per hundred cubic centimeters. The result of the bilirubin excretion test was normal (5 per cent retention). The daily fecal urobilinogen content was 440.0 mg.

In the physical examination of patients with even mild hemolytic icterus, splenomegaly is almost always observed. Otherwise, except for icterus, no pathologic condition may be found. The hematologic findings are of great importance. Two sets of phenomena are to be looked for: (1) those indicating increased hemolytic activity and (2) those indicating increased regenerative activity on the part of the bone marrow. Indicators of increased hemolytic activity are: (1) normal or increased mean corpuscular volume accompanied by microcytosis, (2) increased mean corpuscular thickness, (3) increased thickness and varying degrees of spherocytosis as seen in rouleaux and in fresh isotonic preparations of blood and (4) increased fragility to hypotonic solutions of sodium chloride and to lysolecithin. We have found that

the direct examination of both fresh and stained preparations of blood is of greater value in cases of mild icterus than determination of the mean corpuscular thickness, since the latter may remain practically unaltered although the erythrocytes in fresh preparations of rouleaux

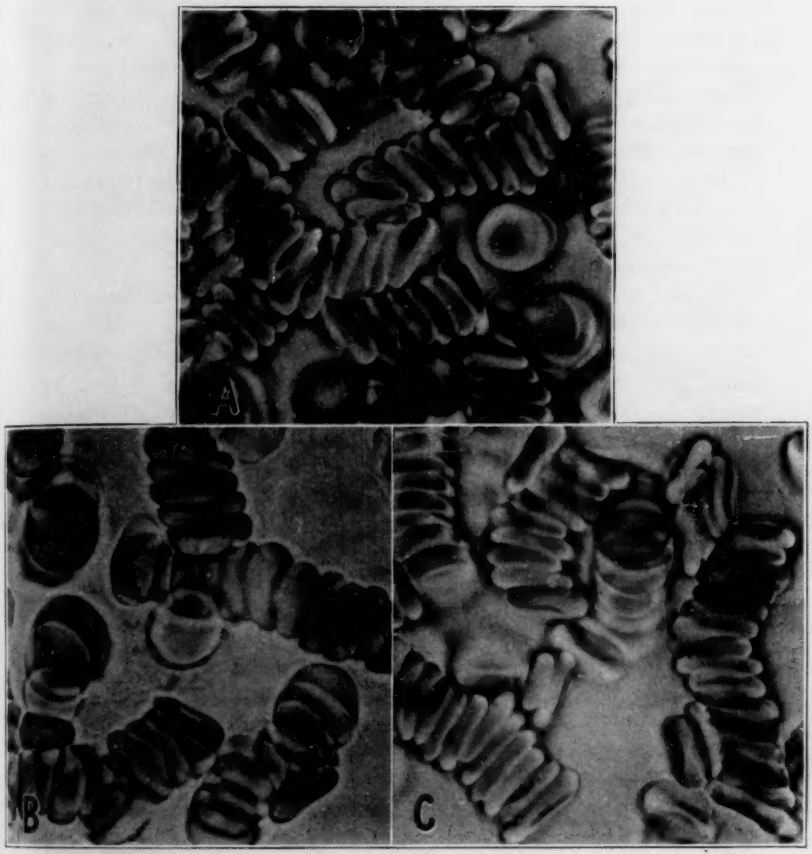

Fig. 3.—Photomicrographs of rouleaux (× 1,000) in fresh preparations of blood. *A*, normal person, normal rouleau formation, with a slight variation in thickness; *B*, R. B., a patient with mild congenital hemolytic icterus, a red cell count of 4,800,000, variation in thickness of individual erythrocytes and a tendency toward increased thickness; *C*, Susie Ru., a patient with familial nonhemolytic icterus, well formed rouleaux and erythrocytes of normal thickness, some apparently even thinner than normal.

and in stained preparations show the characteristic changes of increased thickness. The test for fragility is of even greater importance, since

it frequently reveals abnormality when the figures for thickness are only insignificantly altered. A single examination for fragility may show no definite abnormality; in all cases at least 2 examinations should be made if possible. This was well brought out in the case of Lawrence N., who at different times showed readings which varied from essentially normal (0.48 to 0.16 per cent) to decidedly abnormal (0.72 to 0.04 per cent). The fragility of the red cells to lysolecithin—a lysin derived from normal serum—has been shown by Singer [13] to be abnormal in cases of congenital hemolytic icterus, even when in very mild forms, the fragility to hypotonic solutions of sodium chloride was normal or questionable. This finding has been confirmed by Gripwall.[25] The red cells in 15 cases of familial hemolytic icterus were unusually fragile to solutions of lysolecithin, whereas in all our cases of nonhemolytic icterus in which the patient was examined fragility of the red cells to lysolecithin was normal.

Often just as important as the indicators of increased hemolytic activity are the indicators of increased regenerative activity on the part of the marrow. The latter are reticulocytosis, increased polychromatophilia, polymorphonuclear leukocytosis and thrombocytosis. Except in severe and moderately severe conditions, leukocytosis and thrombocytosis are not particularly evident, but even in the mildest form the reticulocyte count is found to be elevated, at least in serial observations.

Studies on Robert B. and Lawrence N., previously referred to, disclosed that there was great variability in the reticulocyte count, with the percentage varying widely from normal to decidedly abnormal values. On successive counts, abnormal values could always be demonstrated, quite in contrast to the findings in patients with nonhemolytic icterus in whom, even on repeated examinations, a significant elevation in the reticulocyte count was never demonstrated.

The urine in cases of hemolytic jaundice usually shows an increased content of urobilinogen, although normal values may be present even in moderate or severe forms of the disease. Of 30 cases reported by Gripwall,[25] the urobilinogen content of the urine was normal in about one-third. This circumstance was also reported by Watson [26a] and by Barker.[26b] In the cases of nonhemolytic jaundice the content of urobilinogen in the urine was always within normal limits. The outstanding differential

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26a. Watson, C. J.: Studies of Urobilinogen: II. Urobilinogen in the Urine and Feces of Subjects Without Evidence of Disease of the Liver or Biliary Tract, Arch. Int. Med. **59**:196 (Feb.) 1937; III. The Per Diem Excretion of Urobilinogen in the Common Forms of Jaundice and Disease of the Liver, ibid. **59**: 206 (Feb.) 1937.

26b. Barker, W. H.: Excretion of Bile Pigment and Hepatic Function in Diseases of the Blood, Arch. Int. Med. **62**:222 (Aug.) 1938.

feature was the content of urobilinogen in the feces. In 7 cases of congenital hemolytic jaundice, including even the mildest or most latent variety (cf. Robert B.), the daily urobilinogen excretion in the feces was always elevated from 400 to 800 mg. In the cases of nonhemolytic jaundice, on the other hand, the urobilinogen content was either normal or somewhat reduced.

Studies of the urobilinogen content of the feces have not previously been made in instances of familial nonhemolytic icterus. The contrast between the high values obtained for the patients with hemolytic jaundice and the normal or even low values obtained for the patients with non-

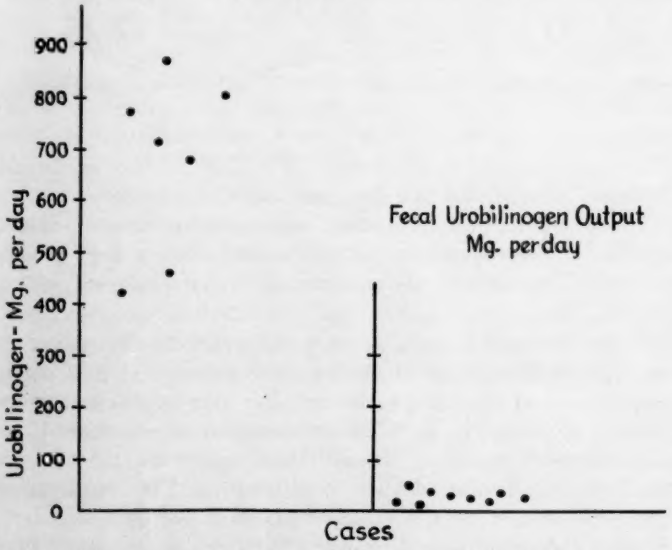

Fig. 4.—The fecal urobilinogen output in 7 cases of familial hemolytic icterus, as compared with that in 9 cases of familial nonhemolytic icterus. Note the low values in the latter cases, as compared with the high values in the former cases.

hemolytic jaundice is striking and serves to rule out all possibility of a hemolytic process, however mild, in the latter.

We have recently demonstrated that the "bilirubin excretion test" may be of value in differentiating cases of mild jaundice of the two types. Thus, in the cases of nonhemolytic jaundice an abnormal type of curve was obtained which indicated delayed excretion of the bilirubin from the blood. Quite in contrast were the normal curves for excretion obtained in cases of mild hemolytic icterus with comparable levels of bilirubin in the blood. (In the case of a patient with severe hemolytic icterus, however, with long-continued destruction of the blood and failure

of response to splenectomy, the bilirubin excretion from the blood was abnormal. This might indicate definite impairment in the function of the hepatic cells.)

The outstanding differential features of the two types are tabulated as follows:

Hemolytic Jaundice	Nonhemolytic Jaundice
Splenomegaly	Normal spleen
Spherocytosis	No spherocytosis
Abnormal thickness of the rouleaux	Normal thickness of the rouleaux
Increased fragility to sodium chloride	Normal fragility to sodium chloride
Increased fragility to lysolecithin	Normal fragility to lysolecithin
Reticulocytosis	Normal reticulocyte count
Increased content of urobilinogen in the urine	Normal content of urobilinogen in the urine
Greatly increased content of urobilinogen in the feces	Normal or low content of urobilinogen in the feces
Normal result of the bilirubin function test	Abnormal (delayed) result in the bilirubin function test

It should be noted that in a given case not all the features mentioned will necessarily be shown. The most constant and reliable are those of splenomegaly, spherocytosis as seen in rouleaux and in stained preparations, reticulocytosis and the amount of urobilinogen excreted in the feces.

B. Differentiation from Chronic Parenchymatous Disease of the Liver: The differentiation of nonhemolytic icterus and mild chronic disease of the liver (hepatitis, early cirrhosis) may be difficult and even impossible, especially in an isolated example of the former disease. Familial hepatitis is rare. Parsons [27] has recently reported cases of hepatitis in a family of 9 children, 4 of whom died of hepatic failure. The parents were not affected. Debré [28] has also reported cases of severe cirrhosis in children of several families. Wilson's disease (hepatolenticular degeneration) may perhaps be included under familial hepatitis. Familial disease does not necessarily signify hereditary disease, since the same toxic or infectious substance might have injured several members of the same family simultaneously. The occurrence of icterus in a family, particularly if at least two generations can be studied, as in the Ru. family, serves to make unlikely the diagnosis of chronic progressive parenchymatous disease and to implicate a simple disturbance in function. However, it must be conceded that the separation of a "functional" abnormality and one due to slight "organic" disease is often difficult.

Chronic hepatitis, although in many cases subject to long remissions, sooner or later shows the evidences of progressive disease. In mild

27. Parsons, F. B.: Familial Hepatitis, Proc. Roy. Soc. Med. **32**:1197, 1939.
28. Debré, R.: Familial Hepatitis and Chronic Jaundice, Lancet **1**:760, 1939.

forms the blood bilirubin may be of the "indirect" variety,[29] and results of all the present day tests for liver function may be entirely normal. Features which may help to differentiate mild true hepatitis from the familial variety presented here are (1) the bilirubin in the urine and (2) the urobilinogen in the urine. However, the results of tests for these substances are subject to much variation and fluctuation. In a series of tests on the urine in a case of mild hepatitis, an occasional specimen may show the presence of bilirubin. Furthermore, the level of urobilinogen in the urine usually becomes increased early in the disease. When a diminished content of urobilinogen is present in the feces in association with hepatitis the stools are usually pasty or even clayey in color. In our familial groups of patients, however, even among those patients with low urobilinogen values, the stools were a normal brown.

In the cases of more severe hepatic disease, the spleen and liver may be enlarged; spider telangiectasias are often found; anemia, often of the macrocytic variety, may be present; "target" cells [30] and decreased fragility to sodium chloride or to lysolecithin may be present; there may be diminution in blood proteins and in cholesterol esters; the bilirubin is usually of the "direct" variety, and at least one of the several types of liver function tests yields an abnormal result.

Nature of Nonhemolytic Icterus and the Possible Pathologic Mechanisms Involved.—Before discussing the possible pathologic mechanisms involved in this condition, it may be desirable to direct attention briefly to the normal physiologic processes involved in the formation and excretion of bilirubin.

The cycle of the conversion of the iron-containing pigment hemoglobin to the iron-free pigment bilirubin has been abundantly studied by numerous investigators.[31] By a series of steps as yet poorly understood, iron is liberated from the hemoglobin of the red cells and the com-

29. That the blood bilirubin is of the "indirect" variety in the early stages or very mild forms of hepatitis, as in the early and convalescent stages of catarrhal jaundice, is not generally appreciated. The mildest disorders of the hepatic cells may not be associated with obstruction to the flow of bile and thus may not show "regurgitation" of "unlinked" bilirubin into the blood stream (Eppinger, H.: Leberkrankheiten, Berlin, Julius Springer, 1937).

30. These are abnormally thin cells which in stained preparations resemble "bull's eyes" or targets. They are unusually resistant to hypotonic solutions of sodium chloride [24] and of lysolecithin.

31. Bingold, K.: Ueber das Schicksal des überalterten Blutfarbstoffes im Organismus, Ztschr. f. d. ges. exper. Med. **99**:22, 1936. Lemberg, R.: The Disintegration of Haemoglobin in the Animal Body, in Perspectives in Biochemistry, London, Cambridge University Press, 1938, p. 137. Rich, A. R.: The Formation of Bile Pigment, Physiol. Rev. **5**:182, 1925. Whipple, G. H.: Pigment Metabolism and Regeneration of Hemoglobin in the Body, Arch. Int. Med. **29**:711 (June) 1922.

pound bilirubin formed. According to Barron [32] and others, bilirubin in the blood stream when first produced is bound to serum protein. This protein linkage is responsible for the "indirect" or "delayed" reaction with the van den Bergh test, since alcohol must be present to "unlink" the bilirubin from its protein combination. This "indirect" bilirubin then passes through the hepatic cells, during which passage an "unlinking" takes place so that the bilirubin then gives an "immediate" or

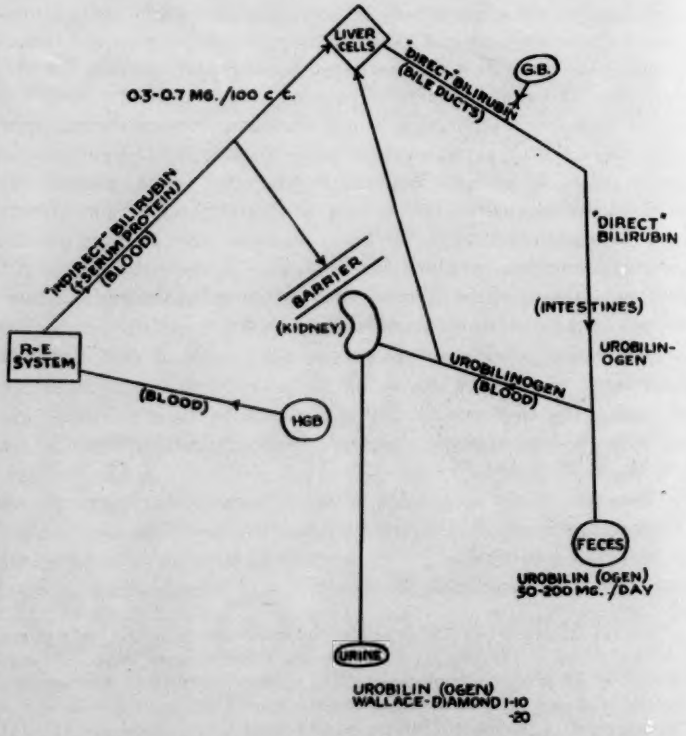

Fig. 5.—Diagrammatic representation of the normal bilirubin metabolism.

"direct" reaction with the same van den Bergh test. Bilirubin is then excreted into the intestine, where it becomes converted into urobilinogen. Some of the latter material is reabsorbed by the intestinal mucosa into the blood stream and excreted by the kidneys into the urine.

Van den Bergh,[3] in his discussion of cases of mild icterus, referred to otherwise healthy persons who had slightly increased values for blood

32. Barron, E. S. G.: Bilirubinemia, Medicine **10**:77, 1931.

bilirubin. He concluded that they represented probable examples of physiologic hyperbilirubinemia. In other words, just as normal persons vary greatly in stature and a person of 76 inches (183 cm.) is considered within the normal range, so it is conceivable that the normal content of bilirubin in the blood may range from 0.3 to 1.0 mg. per hundred cubic centimeters. Some of Meulengracht's cases of the mildest form may have been instances of this type. Because the bilirubin values were usually well above 1.5 mg. per hundred cubic centimeters in our patients, the possibility of simple physiologic bilirubinemia must be discarded.

Rich,[33] in his well known discussion of the pathogenesis of the forms of jaundice, mentioned four conditions under which jaundice may occur in the absence of obstruction: (1) when the threshold of the hepatic cells for bilirubin excretion is greatly raised; (2) when bilirubin is produced more rapidly than the hepatic cells can excrete it; (3) when the excretory mechanism is disturbed to such an extent that the bilirubin produced cannot be satisfactorily removed from the blood, or (4) when any combination of these factors occurs. To these conditions must be added a fifth, postulated by Bauer and Spiegel,[34] i. e., a "paracholic" icterus, in which bilirubin entering the endothelial spaces between the hepatic columns is rapidly reabsorbed into the blood before it can be taken up by the hepatic cells. Aschoff [35] induced such a condition experimentally by tying off the common bile duct. There was at first an increase in "indirect" bilirubin, due in all probability to regurgitation of "unlinked" bilirubin into the endothelial spaces and its subsequent appearance in the blood stream. Similar results were obtained by Lepehne.[35a]

That icterus might be due to an increased threshold of the hepatic cells to excretion of bilirubin was not seriously considered by Rich, who stated that there was no evidence for its existence. Our studies led us to consider this as a possible factor in the pathogenesis in our cases of nonhemolytic icterus. The familial incidence of the condition, its benign nature and its persistence for many years without evidence of progressive hepatic impairment indicated a physiologic or "functional" disturbance rather than a disturbance due to well defined organic disease. A high threshold for the excretion of bilirubin from the blood might reasonably explain the disturbance, especially since this could conceivably

33. Rich, A. R.: The Pathogenesis of the Forms of Jaundice, Bull. Johns Hopkins Hosp. **47**:338, 1930.

34. Bauer, J., and Spiegel, A.: Ueber das Bilirubin im Blute und seine pharmakologische Beeinflussbarkeit, Deutsches Arch. f. klin. Med. **129**:17, 1919.

35. Aschoff, L., cited by Eppinger, H.: Leberkrankheiten, Berlin, Julius Springer, 1937.

35a. Lepehne, G.: Experimentelle Untersuchungen in mechanischen und dynamischen Icterus, Deutsches Arch. f. klin. Med. **136**:88, 1921.

be inherited. Against this possibility was placed the reaction to injected bilirubin. If the "threshold" were fixed, say at 2 mg. per hundred cubic centimeters, the injection of an amount of bilirubin sufficient to raise the bilirubin content of the blood to 4 mg. should be followed by a quick drop in bilirubin to the original value of 2 mg. This did not occur in our cases, in which excretion of bilirubin from the blood stream after intravenous injection of the material was definitely delayed. This delay indicated that the passage of bilirubin through the hepatic cells was retarded and, in the absence of other evidences of disturbed function of the liver, pointed to a selective abnormality in bilirubin excretion by the hepatic cells (retention jaundice).

We believe that the evidence now at hand in our cases points to the presence of a "constitutional hepatic dysfunction" in which the permeability of the hepatic cells to the passage of bilirubin is probably disturbed, with resultant "regurgitation" of this substance first into the endothelial spaces of the liver and thence into the blood stream. In this type of "regurgitant" jaundice, in contrast to that described by Rich, the bilirubin is of the "indirect" type, since it has not passed through the liver cells but is simply retained longer than usual in the blood stream. Histologic examination of liver tissue in our cases was not possible, although Meulengracht (personal communication) stated that he performed a puncture biopsy of the liver in 1 of his cases, with completely negative results.

Toward the close of the present study, we learned of the results of an investigation by Malloy and Lowenstein [36] on a strain of rats which showed hereditary jaundice. The analogies between these families of jaundiced rats and our own human families are striking. Gunn described the mendelian recessive character of jaundice in a mutant strain of Wistar albino rats and suggested the analogy with hemolytic jaundice in human beings. Malloy and Lowenstein found no direct evidence from the blood picture, fragility tests, bone marrow or urobilinogen excretion that the jaundice was primarily of hemolytic origin, although the serum bilirubin was invariably of the indirect type, which is usually considered indicative of hemolytic jaundice.

Their experiments indicated that "the bilirubinemia was due to the inability of the liver to transform indirect to direct bilirubin at a rate sufficient for normal excretion." Indirect bilirubin injected intravenously into jaundiced rats was unchanged in the blood, which demonstrated impairment of excretion in the jaundiced rats. The amount of urobilin excreted in the stool was less than normal, but not entirely absent. Results of other liver function tests, namely, the bromsulphalein and

36. Malloy, H. T., and Lowenstein, L.: Hereditary Jaundice in Rat, Canad. M. A. J. **42**:122, 1940.

Takata-Ara tests and determination of plasma prothrombin levels, were normal in the jaundiced rats. Histologic examination of the liver revealed no morphologic abnormality. It was concluded that the jaundiced rats had retention jaundice caused by the inability of the liver to make direct bilirubin at a rate sufficient for normal excretion.

It appears likely that the hereditary jaundice of rats as described by Malloy and Lowenstein and familial nonhemolytic icterus as described in this paper are similar disorders. It is of interest that the same general conclusions regarding such questions as pathogenesis were independently arrived at in both the investigations on human beings and in those on rats and that histologic examination of the liver in the jaundiced rats failed to reveal any morphologic abnormality. This may indicate a simple disturbance in the function of the liver relating to the excretion of "indirect" bilirubin.

SUMMARY

Two families with an unusual type of chronic hereditary jaundice were studied. Although the bilirubin was of the "indirect" variety, thus suggesting a hemolytic process, the absence of splenomegaly, spherocytosis, reticulocytosis, increased fragility to sodium chloride and lysolecithin and hyperactivity of the bone marrow contraindicated the diagnosis of familial hemolytic icterus. The latter disease was, furthermore, conclusively ruled out by the normal or even somewhat low values for daily urobilinogen output in the feces. The hereditary nature of the disorder and its long duration without symptoms or signs of progressive hepatic disease indicated a simple disturbance in the function of the liver. A significant abnormality was the greatly delayed excretion of injected bilirubin from the blood, indicating (in absence of other evidences of disturbed function of the liver) an alteration in the permeability of the hepatic cells which retarded the excretion of bilirubin. Striking analogies were present between the familial nonhemolytic jaundice in human beings in our cases and the hereditary jaundice of rats described by Malloy and Lowenstein; both conditions may represent a "constitutional hepatic dysfunction" (Rozendaal, Comfort and Snell). Our studies have further demonstrated that familial jaundice is not always hemolytic in type and that an indirect van den Bergh reaction is not necessarily indicative of a hemolytic process.

NOTE.—Since this paper was submitted, another family has been observed. Splenectomy had been suggested for one of the persons in this family because the diagnosis of familial hemolytic jaundice had been made. In addition, 4 young persons with the symptoms, signs and laboratory phenomena in the cases described in this paper were studied; unfortunately, studies of the families of these 4 persons could not be made.

PNEUMOCOCCIC PNEUMONIA

AN ANALYSIS OF THE RECORDS OF 1,469 PATIENTS TREATED IN THE LOS ANGELES COUNTY HOSPITAL FROM 1934 TO 1938 INCLUSIVE

III. TYPE-SPECIFIC ANTIPNEUMOCOCCUS SERUM THERAPY: QUANTITATIVE RELATIONS BETWEEN DOSAGE AND RESULTS

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AND

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LOS ANGELES

Treatment of pneumococcic pneumonia with type-specific antipneumococcus serum has received wide attention.[1] "Adequate" treatment is said to hasten recovery and improve the outcome. Three major

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This study was aided by the Pneumonia Fund of the School of Medicine, University of Southern California.

From the Department of Medicine, School of Medicine, University of Southern California and from the Los Angeles County Hospital.

1. Anders, J. M.: Serum Treatment of Pneumonia, J. A. M. A. **43**:1770-1780 (Dec. 10) 1904. Cole, R.: Treatment of Pneumonia by Means of Specific Serum, ibid. **41**:663-666 (Aug. 30) 1903. Avery, O.; Chickering, H. T.; Cole, R., and Dochez, A.: Acute Lobar Pneumonia: Prevention and Serum Treatment, Monograph 7, Rockefeller Institute for Medical Research, 1917. Huntoon, F. M., and Craig, S. H.: Polyvalent Antibody Response to Multiple Antigens, J. Immunol. **6**:235-247 (March) 1921. Cecil, R. L., and Larsen, N. P.: Clinical and Bacteriologic Study of One Thousand Cases of Lobar Pneumonia, with Special Reference to the Therapeutic Value of Pneumococcus Antibody Solution, J. A. M. A. **79**:343-349 (July 29) 1922. Conner, L. A.: Experiences in New York Hospital with the Treatment of Lobar Pneumonia by a Serum-Free Solution of Pneumococcus Antibodies, Am. J. M. Sc. **164**:832-846 (Dec.) 1922. Felton, L. D.: A Study of the Isolation and Concentration of the Specific Antibodies of Antipneumococcus Sera, Boston M. & S. J. **190**:819-825 (May 15) 1924. Park, W. H.; Bullowa, J. G. M., and Rosenblüth, M.: The Treatment of Lobar Pneumonia with Refined Specific Antibacterial Serum, J. A. M. A. **91**:1503-1508 (Nov. 17) 1928. Cecil, R. L., and Sutliffe, W. D.: The Treatment of Lobar Pneumonia with Concentrated Antipneumococcus Serum, ibid. **91**:2034-2042 (Dec. 29) 1928. Cole, R.: Serum Treatment in Type I Lobar Pneumonia, ibid. **93**: 741-747 (Sept. 7) 1929. Finland, M.: The Serum Treatment of Lobar Pneumonia, New England J. Med. **202**:1244-1247 (June 26) 1930. Cecil, R. L., and

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factors determine the "adequacy" of serum: the duration of the untreated disease, the total amount of serum given and the rapidity of

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its administration. It is reasonable that these factors are interdependent in such a way that the relations between them are quantitative and amenable to analysis.

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MATERIALS AND METHODS [2]

In the preceding articles [3] certain data have been presented concerning all (1,469) patients with typed pneumococcic pneumonia treated in the Los Angeles County Hospital during the five year period 1934 to 1938. Of these, 569 received specific serum therapy, but 56 cases were discarded because the records were inadequate. The remaining 513 are otherwise unselected.

All patients received commercial type-specific, horse or rabbit antipneumococcus serum intravenously, in various hospital services, at the order of many different physicians and in widely varying dosage.

The entire clinical, nursing and laboratory record of each patient was abstracted in detail by some of the group of workers. The abstracts were checked by one of us with the original records, and the data were then analyzed [4] to determine the relation of treatment to outcome and to the defervescence. Certain terms, illustrated in figure 1, have been used throughout this paper.

In studying the relation of treatment to outcome, we used the following terms: *U* is the total number of thousand units of serum received by the patient, *H* is the number of hours between the first and the last dose of serum (with a minimum

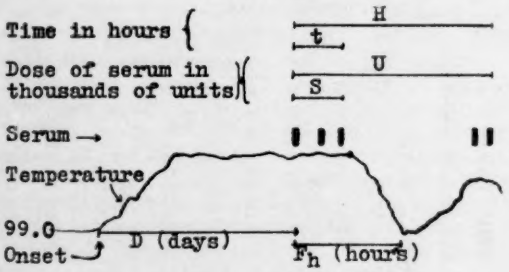

Fig. 1.—Explanation of symbols.

of 1) and *D* is the number of days from the onset (first appearance of chill, fever, painful respiration, bloody sputum, dyspnea or prostration) of pneumonia to the first dose of serum (with a minimum of 1). The relation of treatment to outcome was studied in all (513) patients.

The relation of treatment to the defervescence was studied in the 462 patients whose temperature after serum therapy fell to 99.0 F. or less before cure, death or recognition of a complication. The following terms have been used: *S* is the

2. Dr. Thurston H. Ross, of the School of Merchandizing, University of Southern California, has "examined the statistical method developed in this paper, and found that, though unique, it is adequate to support the findings indicated."

3. Moore, F. J., and others: Pneumococcic Pneumonia: An Analysis of the Records of 1,469 Patients Treated in the Los Angeles County Hospital from 1934 to 1938, Inclusive; I. Character of Pneumonia Caused by the Various Types; Complications of Pneumonia; Outcome of Pneumonia in the Presence of Certain Variations in the Patient and in the Course of the Disease, Arch. Int. Med. **66**: 1290-1316 (Dec.) 1940; II. Outcome and Character of Pneumonia in the Presence of Associated or Concomitant Disease, ibid. **66**:1317-1330 (Dec.) 1940.

4. Mary Cunliffe Moore and Josephine Abraham aided in the conduct of the analysis.

amount of serum, in thousands of units, given before the first normal temperature reading (99.0 F. or less), while t is the time in hours from the first to the last dose of serum given before the first normal temperature reading (minimum of 1); F_h is the number of continuously febrile hours following the first dose of serum, e. g., the number of hours from the first dose to the first normal temperature reading—it implies nothing about the occurrence of subsequent fever. The character of the defervescence (crisis or lysis) and the recurrence of fever (over 99.0 F.) have also been noted.

The outcome has been recorded as "optimum outcome" [5] (survival without complication), "dead" (accidental deaths have been excluded from this study) or "complicated" (as defined in paper I).

Certain aspects of our findings have been found to be more readily understood if their presentation is preceded by an outline of the preliminary findings and the subsequent analysis.

TABLE 1.—*Outcomes Attending Variations in U (total dose in thousands of units), D (number of days from onset to first dose) and H (number of hours from first to last dose)*

Table 1a					Table 1b					Table 1c				
		% Outcome					% Outcome					% Outcome		
U	Pt*	oo†	d‡	c§	D	Pt	oo	d	c	H	Pt	oo	d	c
1-20	15	60	33	7	1	27	96	0	4	1-2	37	70	22	11
21-40	23	70	26	9	2	72	85	3	14	3-4	42	74	17	12
41-60	35	74	23	9	3	73	85	7	12	5-6	40	88	3	12
61-80	33	70	15	21	4	82	71	17	15	7-8	60	85	7	8
81-100	108	79	11	11	5	81	68	19	17	9-10	41	81	17	7
101-120	32	75	9	19	6	57	70	18	18	11-14	49	69	22	16
121-140	63	81	2	17	7	42	48	33	31	15-20	60	73	13	15
141-160	31	84	13	6	8	44	59	27	18	21-30	60	74	13	15
161-180	36	72	0	28	9-11	19	37	37	47	31-50	60	47	22	37
181-200	23	65	13	26	12-31	18	56	33	22	51-100	46	67	22	22
201-220	22	63	22	22						101 & up	21	38	38	48
221-260	31	58	32	16										
261-300	23	61	26	17										
301-400	22	45	32	41										
401 & up	17	41	47	35										

* Number of patients. † Optimum outcome. ‡ Dead § Complicated.

We examined the outcome attending various total dosages (U), expecting to find an improving outcome with increasing dosage. This relation was found (table 1 *a*) only with relatively small amounts of serum, larger amounts being accompanied by an increasingly poor outcome. The anomaly was thought to have been due to either or both of the following facts: (1) Patients receiving the larger doses of serum had much greater need for serum than did those receiving smaller doses, (2) the larger amounts were spread out over such a long period of time that they were ineffective.

The need for serum is well known to increase with the duration of the disease untreated. We found (table 1 *b*) that the outcome was definitely impaired with increasing numbers of days (D) from the onset to the first dose of serum, the dosage not being considered. It was apparent that D might be used as an index

5. Since a patient may die with a complication and hence appear under both headings, there is need of the optimum outcome rate, for it permits calculation of the degree of overlap between the death and complication rates: the sum of the three rates minus 100 equals the degree of overlap. The optimum outcome rate is of great interest per se.

of the need for serum or that division of the total dose by D might give a quotient any value of which was accompanied by a constant outcome (regardless, that is, of the actual value of either U or D).

The number of hours (H) between the first and the last dose of serum was also examined, the expectancy being that the outcome was increasingly poor with increasing values of H. As seen in table 1 *c*, this was actually found except for very small values of H. This intimated that the rate of serum administration, or U/H, should be considered rather than the total dose alone. When U/H was substituted for U, the expression U/D, previously noted, became U/HD, or the amount of serum per hour per day untreated.

When U/HD was studied in a similar manner, it presented a fairly satisfactory correlation with outcome. However, if the patients were divided according to D (days untreated), it was found that patients with similar U/HD but different D values differed considerably in outcome. The criterion that a given value of the expression should be accompanied by a given outcome regardless of the actual value of U, H or D was therefore not met. Certain other expressions were hence examined: U/HD^2; U^2/HD^3; U/HD^3, and similar expressions omitting H. The correlation of U/HD^3 with the outcome was more acceptable than that of the other formulas, and it is presented alone. Since the relation was logarithmic, we have dealt with the logarithm of the expression rather than its numerical value.[6]

To be acceptable, it was also expected that the general form of the relation between U/HD^3 and the outcome should be independent of bacteremia, pulmonary involvement and other factors that may alter the effectiveness of serum. It was not expected that the same outcome should attend the same treatment under those various conditions, but only that parallel alterations in outcome should be found with alterations in U/HD^3. Several factors were examined in this manner.

With regard to the effect of serum on the temperature, an attempt was made to discover a relation between treatment preceding the defervescence and the interval of time between the beginning of treatment and the first normal temperature. The amount of serum per hour, S/t (S being the number of thousand units given before the first normal temperature and t the time in hours between the first and last injection), was plotted against the number of hours the patient was continuously febrile, F_h (the number of hours between the first dose of serum and the first normal temperature reading). The correlation was found adequate, and other formulas were not considered. The relation was examined to determine whether it could have been due solely to S, to the extent of pulmonary involvement or to the incidence of bacteremia. It was more closely examined to determine the effect of the number of days untreated (D) and of those types represented by sufficient numbers to warrant study (I, II, VII, the remainder being pooled).

FINDINGS

Relations Between Dosage and Outcome.—The data showing the relation of U/HD^3 to outcome are presented numerically in table 2, parts 1 to 12, while mean percentages (calculated by the method of least squares) are indicated in graphs (fig. 2) corresponding to the parts of the table.

6. When the $\log_{10}$ of $U/H\ D^3$ equaled zero, the patient received 1,000 units of serum per hour for the cube of the number of days untreated. This obviously implies nothing about the actual value of U, H or D—which is the reason the formula must be proved valid regardless of the actual value of U, H or D.

TABLE 2.—*Relation Between Outcome and U/HD ^{3}, in All (513) Serum-Treated Patients*

The table is composed of twelve parts, each of which gives numerical data and corresponds to a graph giving the data in percentage form (mean per cents, calculated by the method of least squares). Part 1 indicates outcome of all patients grouped according to class intervals of 0.3 mean log U/HD^3, and the actual values of U/HD^3; the remainder of the table indicates the effect of certain other variables. U, total dose in thousands of units; H, number of hours between first and last dose (minimum of 1); D, number of days from onset to first dose (minimum of 1); 00, optimum outcome; d, dead; c, complicated; Tl, total number of patients.

Part 1—All Patients

Mean Log U/HD^3	Actual Numerical Values of U/HD^3	Tl	oo	d	c
−3.6667	0.0002 to 0.0003	2	0	2	0
−3.3333	0.0004 to 0.0007	1	0	1	1
−3.0000	0.0008 to 0.0015	6	4	1	2
−2.6667	0.0016 to 0.0032	7	3	2	2
−2.3333	0.0033 to 0.0068	17	7	4	9
−2.0000	0.0069 to 0.0147	31	17	9	8
−1.6667	0.0148 to 0.0316	74	44	18	18
−1.3333	0.0317 to 0.0681	86	57	22	10
−1.0000	0.0682 to 0.1468	79	54	12	18
−0.6667	0.1469 to 0.3161	55	40	11	5
−0.3333	0.3162 to 0.6813	60	54	2	5
0.0000	0.6814 to 1.4680	39	31	1	8
0.3333	1.4681 to 3.1610	26	23	0	3
1.2856	3.1611 to 200.00	30	30	0	0
Total		513	364	85	89

Part 2a.—U, or Total Dose in Thousands of Units

Mean Log U/HD^3	U 1 to 80				U 81 to 120				U 121 to 200				U 201 and Over			
	Tl	oo	d	c	Tl	oo	d	c	Tl	oo	d	c	Tl	oo	d	c
−3.67	0	..	..	..	1	0	1	0	0	..	..	..	1	0	1	0
−3.33	0	..	..	..	0	..	..	..	0	..	..	..	1	0	1	1
−3.00	1	1	0	0	3	1	1	2	0	..	..	..	2	2	0	0
−2.67	0	..	..	..	2	1	1	0	4	2	0	2	1	0	1	0
−2.33	3	2	1	1	4	1	1	2	7	2	1	5	3	2	1	1
−2.00	9	4	3	3	7	4	2	1	6	5	0	1	9	4	4	3
−1.67	12	7	4	1	20	13	6	1	22	16	1	7	19	8	7	9
−1.33	23	13	7	3	16	15	0	1	20	15	3	2	28	14	12	4
−1.00	17	12	3	3	18	14	2	3	24	16	4	6	20	12	3	6
−0.67	15	10	5	1	17	16	0	1	11	8	1	2	12	6	5	1
−0.33	13	12	1	1	18	16	1	1	17	17	0	0	12	9	0	3
0.00	5	5	0	0	15	12	0	3	17	13	0	4	2	1	1	1
0.33	6	5	0	1	7	5	0	2	10	10	0	0	3	3	0	0
1.29	3	3	0	0	11	11	0	0	13	13	0	0	3	3	0	0
Total	106	74	24	14	139	109	15	17	152	117	10	29	116	64	36	29

Part 2b.—H, or Number of Hours Between First and Last Dose

Mean Log U/HD^3	H 1 to 6				H 7 to 14				H 15 to 30				H 31 and Over			
	Tl	oo	d	c	Tl	oo	d	c	Tl	oo	d	c	Tl	oo	d	c
−3.67	0	..	..	..	1	0	1	0	0	..	..	..	1	0	1	0
−3.33	0	..	..	..	0	..	..	..	0	..	..	..	1	0	1	1
−3.00	0	..	..	..	1	0	1	1	2	2	0	0	3	2	0	1
−2.67	0	..	..	..	0	..	..	..	2	1	1	0	5	2	1	2
−2.33	0	..	..	..	4	2	2	1	4	3	0	1	9	2	2	7
−2.00	4	1	1	2	3	2	1	0	8	4	3	1	16	10	4	5
−1.67	3	2	1	0	17	9	6	2	24	16	4	5	30	17	7	11
−1.33	14	9	4	1	24	19	5	2	23	16	4	3	25	13	9	4
−1.00	16	12	3	3	24	19	2	3	23	16	5	4	16	7	2	8
−0.67	15	10	4	1	24	19	3	3	8	7	0	1	8	4	4	0
−0.33	14	13	1	1	25	22	1	2	13	13	0	0	8	6	0	2
−0.00	15	13	0	2	11	10	0	1	12	8	0	4	1	0	1	1
0.33	11	9	0	2	9	9	0	0	2	2	0	0	4	3	0	1
1.29	16	16	0	0	6	6	0	0	7	7	0	0	1	1	0	0
Total	108	85	14	12	149	117	22	15	128	95	17	19	128	67	32	43

TABLE 2.—*Relation Between Outcome and U/HD [3], in All (513) Scrum-Treated Patients—Continued*

Part 2c.—D, or Number of Days from Onset to First Dose

Mean Log U/HD[3]	D 1 to 3				D 4 to 6				D 7 and Over			
	Tl	oo	d	c	Tl	oo	d	c	Tl	oo	d	c
−3.67	0	..	..	..	0	..	..	..	2	0	2	0
−3.33	0	..	..	..	0	..	..	..	1	0	1	1
−3.00	0	..	..	..	0	..	..	..	6	4	1	2
−2.67	0	..	..	..	0	..	..	..	7	3	2	2
−2.33	0	..	..	..	2	1	1	1	15	6	3	8
−2.00	0	..	..	..	11	8	2	1	20	9	7	7
−1.67	0	..	..	..	39	25	7	9	35	19	11	9
−1.33	3	3	0	0	54	36	13	7	29	18	9	3
−1.00	13	6	2	7	58	44	7	9	8	4	3	2
−0.67	21	18	3	1	34	22	8	4	0	..	..	..
−0.33	43	39	1	3	17	15	1	2	0	..	..	..
0.00	35	29	1	6	4	2	0	2	0	..	..	..
0.33	26	23	0	3	0	..	..	..	0	..	..	..
1.29	30	30	0	0	0	..	..	..	0	..	..	..
Total	171	148	7	20	219	153	39	35	123	63	39	34

Part 3.—Type of Pneumococcus

Log U/HD[3]	Type I				Type II				Type III				Other Types			
	Tl	oo	d	c	Tl	oo	d	c	Tl	oo	d	c	Tl	oo	d	c
−3.67	1	0	1	0	0	..	..	..	1	0	1	0	0	..	..	..
−3.33	1	0	1	1	0	..	..	..	0	..	..	..	0	..	..	..
−3.00	3	1	1	2	1	1	0	0	2	2	0	0	0	..	..	..
−2.67	5	2	1	2	2	1	1	0	0	..	..	..	0	..	..	..
−2.33	8	3	1	5	3	1	1	2	2	2	0	0	4	1	2	2
−2.00	12	7	3	3	2	1	0	1	7	5	0	2	10	4	6	2
−1.67	33	23	5	8	7	4	2	1	18	10	5	5	16	7	6	4
−1.33	33	26	4	4	10	7	2	2	28	16	11	2	15	8	5	2
−1.00	41	28	5	11	5	4	1	1	15	10	2	3	18	12	4	3
−0.67	25	22	2	2	5	1	3	1	12	7	3	2	13	10	3	0
−0.33	25	23	0	2	10	9	1	1	10	10	0	0	15	12	1	2
0.00	17	14	0	3	5	3	1	2	4	4	0	0	13	10	0	3
0.33	9	9	0	0	3	1	0	2	6	6	0	0	8	7	0	1
1.29	11	11	0	0	5	5	0	0	5	5	0	0	9	9	0	0
Total	224	169	24	43	58	38	12	13	110	77	22	14	121	80	27	19

Part 4.—Results of Blood Culture in 142 Patients

Mean Log U/HD[3]	1 or More Positive				All Cultures Negative			
	Tl	oo	d	c	Tl	oo	d	c
−3.67	0	..	..	..	0	..	..	..
−3.33	1	0	1	1	0	..	..	..
−3.00	0	..	..	..	2	1	1	1
−2.67	0	..	..	..	1	1	0	0
−2.33	0	..	..	..	7	3	2	4
−2.00	2	0	2	1	7	5	1	2
−1.67	1	1	0	0	18	7	7	7
−1.33	1	0	1	0	20	11	7	3
−1.00	3	2	0	1	25	18	5	5
−0.67	2	0	2	0	11	9	1	1
−0.33	1	1	0	0	22	18	4	0
0.00	1	0	1	1	6	4	0	2
0.33	0	..	..	..	4	3	0	1
1.29	1	1	0	0	6	6	0	0
Total	13	5	7	4	129	86	28	26

Part 5.—Pulmonary Involvement "Lobar" Includes Clinically Lobar Lesions Found at Autopsy to be Atypical

Mean Log U/HD[3]	Typically Lobar (Clinical Diagnosis)												Distinctly Atypical			
	1 Lobe				2 Lobes				3 to 5 Lobes							
	Tl	oo	d	c	Tl	oo	d	c	Tl	oo	d	c	Tl	oo	d	c
−3.67	0	..	..	..	1	0	1	0	1	0	1	0	0	..	..	..
−3.33	1	0	1	1	0	..	..	..	0	..	..	..	0	..	..	..
−3.00	2	1	0	1	1	1	0	0	2	1	1	1	1	1	0	0
−2.67	2	1	0	1	3	1	1	1	2	1	1	0	0	..	..	..
−2.33	8	5	1	3	2	1	0	1	5	0	2	5	2	1	1	0
−2.00	13	7	5	1	11	7	2	3	4	1	2	3	3	2	0	1
−1.67	40	29	4	8	23	13	6	6	9	1	7	4	2	1	1	0
−1.33	50	39	8	5	16	8	6	3	13	6	6	1	7	4	2	1
−1.00	50	40	2	9	13	8	1	4	10	4	6	3	6	2	3	2
−0.67	37	31	4	3	12	8	3	2	2	1	1	0	4	1	3	0
−0.33	41	38	1	2	10	7	1	3	6	6	0	0	3	3	0	0
0.00	31	26	1	5	5	2	0	3	0	..	..	..	3	3	0	0
0.33	21	20	0	1	4	2	0	2	0	..	..	..	1	1	0	0
1.29	25	25	0	0	2	2	0	0	0	..	..	..	3	3	0	0
Total	321	261	27	40	103	60	21	28	54	21	27	17	35	22	10	4

TABLE 2.—*Relation Between Outcome and U/HD*[3]*, in All (513) Serum-Treated Patients—Continued*

Part 6.—Maximum Reported Total Leukocyte Count, in Thousands of Cells (360 Patients)

Mean Log U/HD[3]	0 to 9				10 to 29				30 and Over			
	Tl	oo	d	c	Tl	oo	d	c	Tl	oo	d	c
−3.67	0	..	..	..	1	0	1	0	0	..	..	..
−3.33	0	..	..	..	1	0	1	1	0	..	..	..
−3.00	0	..	..	..	3	3	0	0	1	0	1	1
−2.67	1	1	0	0	4	1	2	1	2	1	0	1
−2.33	1	0	1	1	11	5	2	5	2	1	0	1
−2.00	2	1	0	1	16	9	5	3	5	3	2	1
−1.67	2	1	1	0	39	24	6	11	7	4	2	1
−1.33	9	5	3	2	49	36	9	4	5	3	2	1
−1.00	7	5	1	2	45	30	6	11	6	5	0	1
−0.67	4	2	2	0	27	21	4	3	2	2	0	0
−0.33	10	9	0	1	26	25	0	1	5	4	0	1
0.00	3	3	0	0	22	16	1	6	7	5	0	2
0.33	4	4	0	0	12	9	0	3	3	3	0	0
1.29	4	4	0	0	11	11	0	0	1	1	0	0
Total	47	35	8	7	267	190	37	49	46	32	7	10

Part 7.—Season

Mean Log U/HD[3]	Winter Dec. to Feb.				Spring March and April				Summer May to Sept.				Fall Oct. and Nov.			
	Tl	oo	d	c	Tl	oo	d	c	Tl	oo	d	c	Tl	oo	d	c
−3.67	2	0	2	0	0	..	..	..	0	..	..	..	0	..	..	..
−3.33	0	..	..	..	0	..	..	..	1	0	1	1	0	..	..	..
−3.00	3	1	1	2	0	..	..	..	3	3	0	0	0	..	..	..
−2.67	5	2	1	2	1	0	1	0	1	1	0	0	0	..	..	..
−2.33	10	3	3	6	3	1	1	2	1	0	0	1	3	3	0	0
−2.00	11	4	4	4	7	3	3	2	5	5	0	0	8	5	2	2
−1.67	44	29	10	8	10	3	4	4	11	6	1	4	9	6	3	2
−1.33	37	21	11	6	12	7	3	3	23	19	4	1	14	10	4	0
−1.00	32	23	4	7	20	12	5	5	14	10	1	4	13	9	2	2
−0.67	26	19	6	1	7	6	0	1	16	12	2	2	6	3	3	1
−0.33	26	24	1	1	16	15	1	1	10	8	0	2	8	7	0	1
0.00	10	9	0	1	9	8	0	1	13	10	0	3	7	4	1	3
0.33	10	8	0	2	9	9	0	0	4	3	0	1	3	3	0	0
1.29	18	18	0	0	4	4	0	0	5	5	0	0	3	3	0	0
Total	234	161	43	40	98	68	18	19	107	82	9	19	74	53	15	11

Part 8.—Age

Mean Log U/HD[3]	0 to 19				20 to 39				40 to 59				60 and Over			
	Tl	oo	d	c	Tl	oo	d	c	Tl	oo	d	c	Tl	oo	d	c
−3.67	0	..	..	..	0	..	..	..	1	0	1	0	1	0	1	0
−3.33	0	..	..	..	0	..	..	..	1	0	1	1	0	..	..	..
−3.00	1	1	0	0	0	..	..	..	4	3	0	1	1	0	1	1
−2.67	0	..	..	..	4	2	0	2	3	1	2	0	0	..	..	..
−2.33	1	0	1	1	10	4	1	6	3	1	1	2	3	2	1	0
−2.00	4	2	0	2	14	8	3	4	10	5	5	2	3	2	1	0
−1.67	6	3	1	2	33	22	6	5	27	16	6	9	8	3	5	2
−1.33	7	6	1	0	42	30	7	6	28	16	10	3	9	5	4	1
−1.00	8	5	0	3	36	28	3	5	24	17	3	7	11	4	6	3
−0.67	11	11	0	0	27	21	4	3	12	6	4	2	5	2	3	0
−0.33	11	10	0	1	25	24	0	1	10	16	1	3	5	4	1	0
0.00	10	8	0	2	21	16	1	5	6	5	0	1	2	2	0	0
0.33	3	3	0	0	12	10	0	2	10	9	0	1	1	1	0	0
1.29	6	6	0	0	18	18	0	0	4	4	0	0	2	2	0	0
Total	68	55	3	11	242	183	25	39	152	99	34	32	51	27	23	7

Part 9.—Race

Mean Log U/HD[3]	Caucasian				Other Races			
	Tl	oo	d	c	Tl	oo	d	c
−3.67	1	0	1	0	1	0	1	0
−3.33	1	0	1	1	0	..	..	..
−3.00	3	2	1	1	3	2	0	1
−2.67	5	2	1	2	2	1	1	0
−2.33	15	7	3	7	2	0	1	2
−2.00	19	8	8	6	12	9	1	2
−1.67	57	38	10	11	17	6	8	7
−1.33	57	36	15	8	29	21	7	2
−1.00	64	43	10	15	15	11	2	3
−0.67	41	30	8	4	14	10	3	1
−0.33	49	47	0	2	11	7	2	3
0.00	29	22	0	7	10	9	1	1
0.33	18	15	0	3	8	8	0	0
1.29	22	22	0	0	8	8	0	0
Total	381	272	58	67	132	92	27	22

TABLE 2.—*Relation Between Outcome and U/HD³, in All (513) Serum-Treated Patients—Continued*

Part 10.—Sex

Mean Log U/HD³	Male				Female			
	Tl	oo	d	e	Tl	oo	d	e
−3.67	1	0	1	0	1	0	1	0
−3.33	1	0	1	1	0	..	..	..
−3.00	3	2	1	1	3	2	0	1
−2.67	6	2	2	2	1	1	0	0
−2.33	9	2	3	6	8	5	1	3
−2.00	22	11	7	7	9	6	2	1
−1.67	52	29	14	14	22	15	4	4
−1.33	59	40	15	7	27	17	7	3
−1.00	58	41	10	12	21	13	2	6
−0.67	38	26	9	4	17	14	2	1
−0.33	36	33	2	2	24	21	0	3
0.00	26	21	1	5	13	10	0	3
0.33	16	15	0	1	10	8	0	2
1.29	22	22	0	0	8	8	0	0
Total	349	244	66	62	162	120	19	27

Part 11.—Maximum Temperature During First Few Hours of Hospitalization

Mean Log U/HD³	99 to 101				102 and 103				104 and Over			
	Tl	oo	d	e	Tl	oo	d	e	Tl	oo	d	e
−3.67	0	..	..	..	2	0	2	0	0	..	..	..
−3.33	0	..	..	..	1	0	1	1	0	..	..	..
−3.00	2	2	0	0	2	1	1	1	2	1	0	0
−2.67	1	1	0	0	3	1	1	1	3	1	1	1
−2.33	1	0	1	0	8	3	1	5	8	4	2	4
−2.00	7	5	2	0	12	6	3	5	12	6	4	3
−1.67	15	9	9	1	38	23	8	11	21	12	5	6
−1.33	14	6	8	1	49	38	8	5	23	13	6	4
−1.00	9	5	4	2	45	33	5	10	25	16	3	6
−0.67	4	4	0	0	33	20	9	5	18	16	2	0
−0.33	3	3	0	0	22	17	2	4	35	34	0	1
0.00	0	..	..	..	23	19	1	4	16	12	0	4
0.33	3	2	0	1	13	12	0	1	10	9	0	1
1.29	2	2	0	0	18	18	0	0	10	10	0	0
Total	61	39	20	5	269	191	42	53	183	134	23	31

Part 12.—Concomitant Conditions

Mean Log U/HD³	Not Recorded ("Absent")				Known to be Present			
	Tl	oo	d	e	Tl	oo	d	e
−3.67	2	0	2	0	0	..	..	..
−3.33	0	..	..	..	1	0	1	1
−3.00	3	2	1	1	3	2	0	1
−2.67	7	3	2	2	0	..	..	..
−2.33	9	4	2	5	8	3	2	4
−2.00	19	11	3	6	12	6	6	2
−1.67	37	25	5	10	37	19	13	8
−1.33	45	33	10	2	41	24	12	8
−1.00	39	27	5	10	40	27	7	8
−0.67	29	24	2	3	26	16	9	2
−0.33	35	30	1	5	25	24	1	0
0.00	18	15	0	3	21	16	1	5
0.33	13	10	0	3	13	13	0	0
1.29	18	18	0	0	12	12	0	0
Total	274	202	33	50	259	162	52	39

Table 2, part 1, and figure 2, graph 1, indicate that the outcome was a function[7] of the amount of serum per hour per day (cubed) untreated. In general, the percentage outcome equaled $a + b$ ($\log_{10}$ U/HD³), in which formula a and b are constants for each index of

7. A variable y is said to be a function of a variable x if there exists a law whereby, when x is given, y is determined. The fact that a definite relation exists is of the first importance; the general statement of the law is less important; the numerical values obtained are relatively inconsequential.

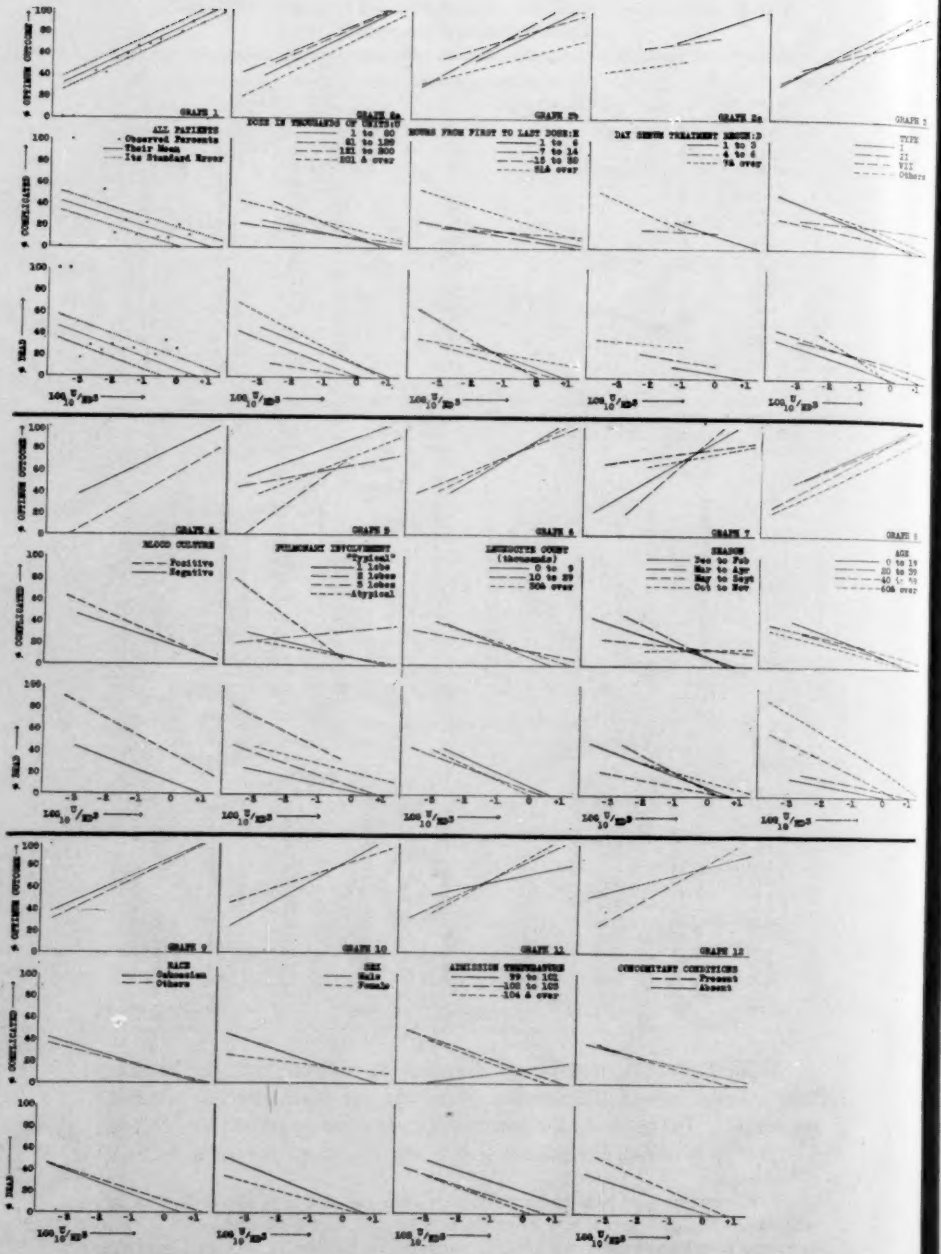

Fig. 2.—Relation between outcome and U/HD ^{3}, in all (513) serum-treated patients. Each of the twelve graphs presents, in percentage form, numerical data from the correspondingly numbered part of table 2.

outcome, U is the total dose of serum in thousands of units, H is the number of hours between the first and the last dose of serum and D is the number of days from the onset of pneumonia to the first dose of serum (days the patient was untreated). The following are the equations drawn in figure 2, graph 1, the standard errors being indicated by plus or minus variations about a:

(1) % Optimum Outcome $= (84.7 \pm 5.8) + 14.86 (\log_{10} U/HD^3)$;

(2) % Complicated $= (9.1 \pm 9.0) - 8.98 (\log_{10} U/HD^3)$, and

(3) % Dead $= (6.7 \pm 11.3) - 10.67 (\log_{10} U/HD^3)$.

As outlined above, it is necessary to know whether these relations were valid for all observed values of U. D and H. Data are presented in table 2, parts 2 *a*, 2 *b* and 2 *c*, and their corresponding graphs. It is seen that the formula is not ideal since patients with the same value of U/HD^3 but different values of, say, U did not have an identical outcome. A second weakness of the data is the slight overlap, in values of the formula, between patients grouped according to their D values.

Data concerning the possible influence of certain other factors on these relations are indicated in table 2, parts 3 to 12, and their corresponding graphs. The results of blood culture, the extent and character of the pulmonary involvement, and the age each influenced one or more of the indexes of outcome, the quality of the alteration being in agreement with expectancy (e. g., the mortality was greater with positive than with negative blood cultures). The values of the constants a and b hence varied, but the general equation remained valid.[8] Smaller differences in the values of a and b were observed with differences in type, season and admission temperature. Very slight or no differences were observed with differences in total leukocyte count, race, sex or the presence or absence of known concomitant conditions. Two anomalies of the complication rate are within statistical expectancy and do not vitiate the otherwise uniform results observed.

As further indexes of outcome, the duration of hospitalization and the total time the patient was febrile (from onset to the last temperature, in the absence of complication, over 99.0 F.) were also studied, but no relation could be established between them and the expression U/HD^3.

Relations between Dosage and the Defervescence.—As shown in table 3 and figure 3, the number of febrile hours (F_h), between the first dose of serum and the first normal temperature reading, was a function of the rate (S/t, thousands of units per hour) of serum administration prior to the first normal temperature. Since this part of the study was

8. A much greater number of patients would permit quantitative studies of these factors by the method here used for D, but that is impossible with the number available in this study.

confined to the 462 patients who had a defervescence before cure, death or the recognition of a complication, the findings cannot be interpreted as indicating that a defervescence will follow a certain treatment; they indicate only that if a defervescence does occur it may be expected at a given time. On the graph is indicated a line, y′, which represents the mean $\log_{10} F_h$ calculated by the method of least squares for the various values of $\log_{10} S/t$. The equation for this line (e. g., for the relation

TABLE 3.—*Relation Between Dosage and the Temperature Response, in the Four Hundred and Sixty-Two Patients Who Became Afebrile (99) Following Serum and Before Termination of the Disease by Cure, Death or Recognition of a Complication*

Patients are divided according to various rates of serum administration (thousands of units per hour: S/t) before the first normal temperature reading. Part 1 indicates the number of patients and their average duration of continuous fever (Fh), as well as the average amount of serum (S, in thousands of units) received before the defervescence, the average number of lobes involved and the incidence of bacteremia in patients from whom cultures were taken. Part 2 indicates the influence of the duration (in days, D) of untreated illness and of type (I, II, VII, and all others) on the relation between the rate of serum treatment and the duration of continuous fever.

Part 1

Log (S/t)	Total Number of Patients	Log of Average Fh	Average S	Average Number of Lobes	Blood Cultures, % Positive
—0.2218 to 0.6070	55	1.9345	130	1.8	6
0.6071 to 0.9564	118	1.7324	113	1.5	0
0.9565 to 1.1604	104	1.5185	115	1.4	7
1.1605 to 1.2889	39	1.5051	150	1.3	14
1.2890 to 1.3882	44	1.3424	91	1.4	8
1.3882 to 1.6941	86	1.2788	148	1.5	7
1.6942 to 2.3010	16	1.0414	150	1.5	0

Part 2

	Log (Average Fh)			Log (Average Fh)			
Log (S/t)	D 1 to D 3	D 4 to D 6	D 7 and Up	Type I	Type II	Type VII	Other Types
—0.2218 to 0.6070	1.81	1.97	2.06	1.89	2.02	1.82	2.08
0.6071 to 0.9564	1.76	1.73	1.72	1.70	1.64	1.81	1.85
0.9565 to 1.1604	1.53	1.52	1.51	1.54	1.40	1.54	1.46
1.1605 to 1.2889	1.30	1.40	1.88	1.63	1.54	1.30	1.23
1.2890 to 1.3882	1.46	1.38	1.00	1.23	1.23	1.67	1.95
1.3883 to 1.6941	1.20	1.32	1.20	1.32	1.62	1.32	1.04
1.6942 to 2.3010	1.00	1.11	0.78	0.48	1.46	0.78	1.00

between the duration of continuous fever and the rate of serum treatment) is, with the standard error indicated as a plus or minus variation,

$$(4)\ \log_{10} F_h = (2.1842 - 0.3621) - 0.7470\ (\log_{10} S/t).$$

Table 3 presents the following data: the number of patients; the logarithm, to the base 10, of the average F_h; the average value of S; the average number of lobes involved, and the incidence of bacteremia (in studied cases) for various values of the logarithm, to the base 10, of S/t. It is seen that the relation demonstrated in the graph cannot be explained on the basis of the actual dosage (S), of the extent of pulmonary involvement or of the incidence of positive blood cultures. The second part of the table shows that the relation demonstrated in the graph was

independent of the duration of the untreated disease (D) and probably independent of the type of pneumococcus (so far as this was investigated).

The recurrence of fever and the character of the defervescence were also studied. It was found that the incidence of crisis increased with increasing values of S/t, but the incidence of recurrence of fever was virtually constant for all values of S/t. These aspects of the temperature response were also unaltered by the duration of the untreated disease (D).

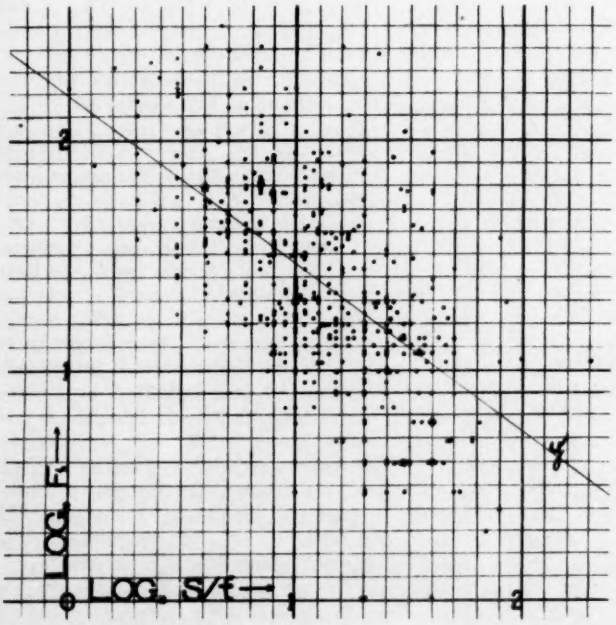

Fig. 3.—Relation between dosage and the temperature response. *S*, number of thousand units of serum given before the first normal temperature; *t*, time in hours from the first to the last dose of that serum; F_h, number of febrile hours from the first dose of serum to the first normal temperature reading. Each point represents one patient. The line, *y'*, is the mean $\log_{10} F_h$ calculated by the method of least squares.

COMMENT

Bearing in mind certain limitations of the data that have been mentioned, one can clearly see from the findings that the outcome was a function of the rate of serum treatment divided by the cube of the number of days the patient was untreated (e. g., U/HD^3), while the speed with which a defervescence followed the initiation of treatment

was a function of the rate of serum treatment (e. g., S/t) without regard to the number of days the patient was untreated. The incompatibility of these findings is superficial.

Serum reacts specifically with materials produced by the pneumococci in the lung; these reactions occur almost exclusively, however, in the blood stream.[9] There may be various kinds of antigen,[10] but the quantitatively predominant one is the specific soluble substance, a haptene derived from the capsule of the organism. This haptene is probably

9. Drinker, C. K.; Enders, J. F.; Shaffer, M. F., and Leigh, O. C.: Emigration of Pneumococci Type III from Blood into Thoracic Duct Lymph of Rabbits, and Survival of These Organisms in Lymph Following Intravenous Injection of Specific Antiserum, J. Exper. Med. **62**:849-860 (Dec.) 1935.

10. Neufeld, F.: Ueber die Agglutination der Pneumokokken und über die Theorien der Agglutination, Ztschr. f. Hyg. u. Infektionskr. **40**:54-72, 1902. Neufeld, F., and Handel, L.: Weitere Untersuchungen über Pneumokokken-Heilsera, Arb. a. d. k. Gsndhtsamte **34**:293-304, 1910. Dochez, A. R., and Gillespie, L. G.: A Biologic Classification of Pneumococci by Means of Immunity Reactions, J. A. M. A. **61**:727-732 (Sept. 6) 1913. Dochez, A. R., and Avery, O. T.: Varieties of Pneumococcus and Their Relation to Lobar Pneumonia, J. Exper. Med. **21**:114-132, 1915. Avery, O. T.: A Further Study of the Biologic Classification of Pneumococci, ibid. **22**:804-819, 1915. Lister, F. S.: Specific Serological Reactions with Pneumococci from Different Sources, Publication 3, South African Institute of Medical Research, Johannesburg, W. E. Hortor & Co., 1914. Heidelberger, M., and Avery, O. T.: The Specific Soluble Substance of Pneumococcus, J. Exper. Med. **38**:73-79 (July) 1923. Avery, O. T., and Heidelberger, M.: Immunological Relationships of Cell Constituents of Pneumococcus, ibid. **38**:81-85 (July) 1923. Heidelberger, M., and Avery, O. T.: The Soluble Specific Substance in Pneumococcus, ibid. **40**:301-316 (Sept.) 1924. Avery, O. T., and Heidelberger, M.: Immunological Relationships of Cell Constituents of Pneumococcus, ibid. **52**:367-376 (Sept.) 1925. Julianelle, L. A., and Reimann, H. A.: The Production of Purpura by Derivatives of the Pneumococcus: I. General Considerations of the Reaction, ibid. **43**:87-95 (Jan.) 1926. Mair, W.: Purpura Producing Substance in Pneumococcus and Heritable Susceptibility of Mice, J. Path. & Bact. **31**:215-219 (April) 1928. Cooper, G.; Edwards, M., and Rosenstein, C.: Separation of Types Among Pneumococci Hitherto Called Group IV and Development of Therapeutic Antisera for Those Types, J. Exper. Med. **49**:461-474 (March) 1929. Pittman, M., and Falls, T. S.: Studies on Respiratory Diseases: Some Relations Between Extracts, Filtrates, and Virulence of Pneumococci, J. Bact. **19**:327-361 (May) 1930. Enders, J. F.: Type Specific Substance Distinct from Specific Carbohydrate in Pneumococcus Type I, J. Exper. Med. **52**:235-252 (Aug.) 1930. Tillett, W. S.; Goebel, W. F., and Avery, O. T.: Chemical and Immunological Properties of Species-Specific Carbohydrate of Pneumococci, ibid. **52**:895-900 (Dec.) 1930. Wadsworth, A., and Brown, R.: Chemical and Immunological Studies of Pneumococcus: Specific Antigenic Carbohydrate of Type I Pneumococcus, J. Immunol. **21**:245-253 (Sept.) 1931. Goodner, K.: Development and Localization of Dermal Pneumococcic Lesion in Rabbit, J. Exper. Med. **54**:847-858 (Dec.) 1931. Cooper, G.; Rosenstein, C.; Walter, A., and Peizer, L.: Further Separation of Types Among Pneumococci Hitherto Included in Group IV and Development of Thera-

distributed throughout the body [11] but is in greatest concentration at the site of infection.[12] Though probably not toxic itself, its relatively great concentration and its avidity for immune bodies cause it to act as a buffer between the natural defenses of the body and the toxic substances or the organisms themselves,[13] which the defenses must destroy to insure the survival of the patient. The specific soluble substance might be called the glue binding the disease to the patient.

By demonstrating that there was a logarithmic diminution in the effectiveness of serum with increasing time illness was untreated, we have not directly proved that there was a logarithmic increase in the need for serum. This would, however, be a plausible deduction. Furthermore, the materials with which serum reacts are formed by organisms whose metabolism is probably a function of their growth curve. Since the latter is logarithmic in vitro, some sort of logarithmic increase in the products of metabolism should be expected.

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peutic Antisera for These Types, ibid. **55**:531-554 (April) 1932. Gundel, M., and Schwarz, F. K. T.: Ueber die Typendifferenzierung und Epidemiologie der Gruppe X der Pneumokokken, Ztschr. f. Hyg. u. Infektionskr. **113**:498-522, 1932. Goodner, K.: Effect of Pneumococcus Autolysates upon Pneumococcus Dermal Infection in Rabbits, J. Exper. Med. **58**:153-160 (Aug.) 1933. Avery, O. T., and Goebel, W. F.: Chemoimmunological Studies on Soluble Specific Substance of Pneumococcus: Isolation of Acetyl Polysaccharide of Pneumococcus Type I, ibid. **58**:731-755 (Dec.) 1933. Oram, F.: Pneumococcus Leucocidin, J. Immunol. **26**: 233-246 (March) 1934. Todd, E. W.: Comparative Serological Study of Streptolysins Derived from Human and Animal Infections, with Notes on Pneumococcal Haemolysin, Tetanolysin, and Staphylococcus Toxin, J. Path. & Bact. **39**:299-321 (Sept.) 1934.

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12. Graeser, J. B.: Specific Soluble Substance of Pneumococcus in Lungs of Dogs Recovered from Experimental Lobar Pneumonia, Proc. Soc. Exper. Biol. & Med. **32**:386-389 (Nov.) 1934. Nye, R. N., and Harris, A. H.: Viable Pneumococci and Pneumococcic Specific Soluble Substance in Lungs from Cases of Lobar Pneumonia, Am. J. Path. **13**:749-767 (Sept.) 1937.

13. Sia, R. H. P.: Studies on Pneumococcus Growth Inhibition: Specific Effect of Pneumococcus Soluble Substance on Growth of Pneumococci in Normal Serum-Leukocyte Mixtures, J. Exper. Med. **43**:633-645 (May) 1926. Wadsworth, A. B., and Sickles, G. M.: Effect of Type I Pneumococcus Culture Broth on Phagocytic Action of Type I Pneumococcus Serum, J. Immunol. **14**:321-328 (Dec.) 1927. Sickles, G. M.: Further Observations on Effect of Type I Pneumococcus Culture Broth on Phagocytic Action of Type I Pneumococcus Serum, ibid. **14**:329-336 (Dec.) 1927. Ward, H. K.: Observations on Phagocytosis of Pneumococcus by Human Whole Blood: Normal Phagocytic Titre, and Anti-Phagocytic Effect of Specific Soluble Substance, J. Exper. Med. **51**:675-684 (May) 1930. Boerner, F., and Mudd, S.: Determination of Phagocytic Power of Whole Blood or Plasma-Leukocyte Mixtures for Clinical or Experimental Purposes, Am. J. M. Sc. **189**:22-35 (Jan.) 1935.

Although the production of fever is probably a complex mechanism, we shall assume, for the sake of simplicity, that in this instance it is activated by the presence in the blood stream of substances with which serum may react specifically. The fact that the relation between treatment and the speed with which a defervescence followed its initiation was independent of the duration of untreated illness requires that the average concentration of those substances (with which serum reacts) must be virtually constant throughout the disease.[14]

At first glance, then, it may seem impossible that the total amount of material—largely specific soluble substance—should increase logarithmically while the blood concentration remained virtually constant. This incompatibility is resolved, however, by the assumption of a threshold mechanism regulating the blood concentration, in conjunction with a diminishing rate of absorption from the lung.[15]

One may conclude that there are definite laws governing the effectiveness of serum treatment as regards outcome and the defervescence and that they have a rational foundation in theory.

Application of these laws to the treatment of the patient requires that their quantitative statement (as in the four equations that have been given) be derived from study of larger numbers of patients than available in this investigation. Certain generalizations, however, may be made at this time. It is probable that excellent results may be expected if the patient is given, for the cube of the number of days untreated, about 10,000 units of serum *per hour,* the duration of treatment being regulated by the response of the patient, e. g., 270,000 units *per hour* for a patient treated on the third day of his illness. Such doses may exceed the amounts one can actually give. One tenth to one one-hundredth of that dose might give good results in certain patients, but one one-thousandth or less would probably be of little if any benefit. It must be noted that there is no statement of the total desired dose. We feel that this cannot be estimated; rather, a definite amount *per hour* should be given until the desired effects are seen. The early defervescence that may follow the larger doses does not necessarily indicate that the patient's stores of antigen have been exhausted but indicates only that serum was administered more rapidly than the blood level of antigen could be restored by absorption from the reservoir in the lung. Hence if after the defervescence a relapse leads to the resumption of treatment, attention should be paid to the fact that the value of H has been increasing during the

14. Nonspecific temperature reactions usually occur almost immediately after treatment is begun, and for this reason we think that the reactions here noted are specific rather than nonspecific.

15. Wang, T. T., and Van Allen, C. M.: Concentration of Congo-Red in Blood After Absorption from Pneumonic Lung, Proc. Soc. Exper. Biol. & Med. **30**:814-816 (March) 1933.

untreated pause; unless extra amounts of serum are given, equal to the amount that would have been given during the interruption of treatment, the numerical value of the expression U/HD^3 will be decreased.

SUMMARY

The effects of type-specific serum on the outcome of pneumococcic pneumonia have been studied in 513 treated patients, unselected except as regards adequacy of their clinical records. The outcome was found to be a function of the treatment, according to the general equation

$$\% \text{ Outcome} = a + b\ (\log_{10} U/HD^3)$$

in which *a* and *b* are constants, U is the total dose in thousands of units of commercial antipneumococcus serum, H is the number of hours between the first and the last dose of serum and D is the number of days from the onset of pneumonia to the first dose of serum. The values of *a* and *b* varied depending, of course, on the index of outcome under consideration—death, complication or survival without complication. Minor and expected variations also occurred with variations in type, pulmonary involvement, bacteremia et al, but the general statement of the law was valid under all circumstances.

The ability of serum to hasten the defervescence was studied in all (462) patients who had a defervescence between the first dose of serum and the recognition of a complication or termination of the disease. The speed with which a defervescence followed initiation of treatment was found to be a function of the rate of that treatment, according to the general equation

$$\text{Log}_{10} F_h = a + b(\log_{10} S/t)$$

in which *a* and *b* are constants, F_h is the number of febrile hours from the first dose of serum to the first normal temperature, S is the amount of serum in thousands of units given before the first normal temperature and t is the time in hours from the first to the last dose of that serum. This relation was found to be independent of the duration of untreated illness, type, pulmonary involvement and bacteremia.

The theoretic and the practical significance of the findings are mentioned.

Dr. J. Leonard Moore assisted in the preparation of this paper.

STUDIES ON OLD AGE PNEUMONIA

II. PROPHYLACTIC EFFECT OF PNEUMOCOCCUS POLYSACCHARIDE AGAINST PNEUMONIA

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The purpose of this paper is to report on work done since 1937 on active immunization with antigenic polysaccharides against pneumonia. The group immunized were inmates of the New York City Home. Most of them were above the age of 50 and a great majority between the ages of 60 and 80 (table 1). There were several reasons why this group was chosen for the experiment. First: They have a high incidence of pneumonia, mortality and case fatality rate. Second: Repeated attacks of pneumonia occur frequently. Third: They could be kept under observation for years, as the turnover in the Home is limited and those who were discharged could be followed up; moreover a great many of them were readmitted to the Home and further observed. Fourth: Those who contracted pneumonia were transferred to the supervising hospital of the New York City Home for Dependents (which was previously the Central and Neurological Hospital and at present is the Welfare Hospital), where they could be clinically studied.

HISTORY AND LITERATURE OF PNEUMOCOCCUS POLYSACCHARIDES

Kraus [1] and Auld [2] discovered almost simultaneously in 1897 the presence of specific precipitable substances in the germ-free culture filtrates of certain bacteria: Kraus in cultures of material from persons with cholera, typhoid fever and bubonic plague and Auld in pneumococcus cultures. The latter also proved that these substances have antigenic properties. Neufeld [3] (1902) showed that the bile solutions

From the II Medical Division and Pathological Laboratories of Welfare Hospital.

This work was started at the Central and Neurological Hospital and the New York City Home for Dependents.

1. Kraus, R.: Wien. klin. Wchnschr. **10**:736, 1897.
2. Auld, A. C.: Brit. M. J. **1**:775, 1897.
3. Neufeld, F.: Ztschr. f. Hyg. u. Infectionskr. **40**:54, 1902.

of pneumococcus cultures give similar results, and Wadsworth [4] (1903) reported the same thing in regard to the filtrates of those organisms in salt solutions.

Dochez and Avery [5] (1917) in their report about the specific soluble substance showed that the presence of this substance is not due to bacterial death and disintegration but represents the extrusion into the medium of bacterial substances during the life process of the organism. They also demonstrated the presence of the specific soluble substance in the serum and urine of patients with pneumonia and showed that its presence in abundance indicates a grave prognosis.

Heidelberger with Avery [6] (1923) studied the chemical constituents of these substances in the different types of pneumonia and showed that the combination of the carbon, hydrogen and oxygen to form varying percentages of carboxyl, acetyl and other radicals and the combination of these groups with the different sugars determine the specificity of the types of pneumococci. Type I has nitrogen and has acidic and basic properties, and they found that it had no antigenic effect. Type II is dextrorotatory and weakly acid and has no nitrogen. Type III is levorotatory and strongly acid and has no nitrogen. Heidelberger and Kendall [7] (1935) worked out quantitative methods for the determination of the amount of precipitable substances from cultures of pneumococci of type III by means of therapeutic serum. The same authors with Scherp [8] (1936) further analyzed the physicochemical qualities of the polysaccharide. They showed that it is thermolabile, and this characteristic may be explained as a partial depolymerization. On the basis of analytic data, these authors assumed that chemically it is a trisaccharide unit for the SI molecule, containing two molecules of uronic acid (possibly both galactouronic) and two atoms of nitrogen.

Avery and Goebel [9] (1933) described an acetyl polysaccharide of the pneumococcus, which, contrary to Heidelberger and Avery's, was antigenic for mice.

From these and other pertinent discoveries it seemed apparent that the cell body of the pneumococcus contains the species-specific nucleoproteins while the capsule contains the type-specific carbohydrates. Both have antigenic properties, but the nucleoproteins react with all

4. Wadsworth, A.: J. M. Research **10**:228, 1903.

5. Dochez, A. R., and Avery, O. T.: J. Exper. Med. **26**:477, 1917.

6. Heidelberger, M., and Avery, O. T.: J. Exper. Med. **38**:73, 1923.

7. Heidelberger, M., and Kendall, F. E.: J. Exper. Med. **61**:559 and 563, 1935.

8. Heidelberger, M.; Kendall, F. E., and Scherp, H. W.: J. Exper. Med. **64**: 559, 1936.

9. Avery, O. T., and Goebel, W. F.: J. Exper. Med. **58**:731, 1933.

varieties (group antigens) while the capsular polysaccharides precipitate only the specific type of pneumococcus from which they are extracted. They are partial antigens or "haptens" in Landsteiner's [10] or "residual antigens" in Zinsser's [11] meaning.

A further step in the investigation of these substances was made when Francis and Tillett [12] (1930) noted that intracutaneous injections of polysaccharides produced not only a homologous but a heterologous stimulation of antibodies.[12a] This heterologous action was confirmed by Finland and Sutliff [13] (1932) and by Felton, Sutliff and Steele [14] (1935).

Felton [15] (1932) also isolated a water-soluble substance from pneumococci of types I and II, which was a fraction apparently nonpolysaccharide and probably nonprotein, contained no acetyl radical and still was antigenic for mice. Later he used various methods for the isolation of the specific soluble substances, precipitating them repeatedly with large volumes of alcohol, some after alkaline treatment and others from the acid-soluble fraction as in the original Heidelberger and Avery method. He also used calcium phosphate for precipitation. His purification procedure was to dissolve the crude product at 0 C. at p_H of 3 or less or even in concentrated hydrochloric acid, or in 1:1 sulfuric acid and repeated precipitation with large amounts of alcohol. The final product contained various amounts of dextrose and nitrogen and was similar in chemical characteristics to the polysaccharide of Heidelberger and Avery. Felton reported that he obtained immunity in white mice and in a small number of human beings.

Felton in collaboration with Ekwurtzel, Simmons and Dublin [16] (1938) reported four experiments on active immunization with this substance in the CCC. camps. The first three experiments were not considered sufficiently controlled to warrant any definite conclusion. In the fourth experiment inoculations were done on 10,740 men in New England and 18,494 in West Coast camps. Since the time of their experiments was short (in some cases sixty-eight and three-tenths days and in others one hundred seventeen and four-tenths days), they took into consideration not only the number of men but also the time of

10. Landsteiner, L.: New England J. Med. **215**:1199, 1936.

11. Zinsser, H., and Parker, J. T.: J. Exper. Med. **37**:275, 1923.

12. Francis, T., and Tillett, W. S.: J. Exper. Med. **52**:573, 1930.

12a. They explained that on the basis of previous pneumococcic infections.

13. Finland, M., and Sutliff, W. D.: J. Exper. Med. **55**:853, 1932.

14. Felton, L. D.; Sutliff, W. D., and Steele, B. F.: J. Infect. Dis. **56**:101, 1935.

15. Felton, L. D.: J. Immunol. **23**:405, 1932.

16. Felton, L. D.; Ekwurtzel, G. M.; Simmons, J. S., and Dublin, L. I.: Pub. Health Rep. **53**:1877, 1938.

exposure. The opportunity for infection for a group of persons is proportional to the product of the number of persons and the number of days each is exposed during the period considered. When this product is divided by 365, the result can be expressed as "years of life exposed to the risk of infection." The years of life exposed were 3,461 in the West Coast camps and 3,153 for the New England camps. The incidence rate in the West Coast camps was 1.73 in the inoculated against 15.69 in the noninoculated group per thousand years of life. The New England camps showed an incidence of 4.34 cases in the inoculated group against 7.28 in the noninoculated. In other words, the incidence rate was about nine times higher in the control group than in the inoculated in the West Coast camps, while in the New England camps it was only about twice as high. According to the report of Felton and his co-workers, "the difference in case ratio between inoculated enrollees in the New England camps and those in the West Coast camps cannot be definitely accounted for." Owing to the circumstances inherent to their experiments, the number of deaths was considered too few to justify any conclusions with regard to the effect of the antigen on the mortality or case fatality rates.

MATERIALS USED IN THIS EXPERIMENT

The antigen which we used was a capsular polysaccharide prepared as follows:[17] Broth cultures of type I and type II pneumococci are centrifuged for forty-eight hours. The supernatant fluid is passed through ultrafiltration. The soluble specific substance is precipitated by the repeated use of large volumes of alcohol in a reaction neutral to slightly acid. The soluble carbohydrates are then tested for their purity and antigenic properties. The material employed was a combination of type I and type II polysaccharides. Chemically the dextrose and nitrogen content was approximately that of the specific soluble substance of Heidelberger and Avery.

METHOD OF THE EXPERIMENT

First Year.—In the winter of 1937-1938, 1,000 persons were immunized with 0.5 cc. of this solution containing 1 mg. of each active substance, and 1,120 persons were kept as controls. The injection of the antigen was given subcutaneously in the deltoid region. The previous history of each person was taken with special reference to antecedent pneumonia or infections of the upper respiratory tract. On every twentieth person the precipitin and mouse-protective value of the blood was determined before immunization.[18] The preliminary work included quantitative and qualitative blood counts on these members of the immunized group. We had

17. The antigen used was prepared first by ourselves and later by a commercial laboratory. At present, through the courtesy of Dr. Felton a type I, II, III antigen—as prepared in the United States Public Health Service Laboratory, Washington, D. C.—is used.

18. Reports on the immunologic studies of these experiments will be published elsewhere.

records of previous physical examinations, temperature, pulse rates and blood pressure for every immunized person. When it seemed necessary, however, physical examinations were given before immunization.

The test for precipitin and mouse-protective value of the serums of the immunized was repeated after eight days, after twenty-one days and after three to four months.

Protocols were kept of both the immunized and the control group, in which the aforementioned data, reactions after immunizations and further information, as to contraction of pneumonia or other diseases were recorded. Persons who left the institution were further observed by a follow-up system.

The hospital record of every patient with pneumonia showed whether he belonged to the immunized or to the control group. Special attention was paid to such patients with regard to time, extension and duration of pneumonia.

The period of observation was continuous from Nov. 1, 1937 to November 1939.

Second Year.—In 1938-1939 the same method was used except that while in the first year the inoculated persons volunteered for the injections, now they were taken as follows: Five hundred new patients were divided in such a way that every other one was chosen for the inoculation and the alternates were kept as controls.

TABLE 1.—*Age Distribution of the Inoculated and Control Groups*

Age Distribution	Inoculated Group on Basis of 1,750		Control Group on Basis of 1,870	
	Number of Persons	Per Cent	Number of Persons	Per Cent
Under 40 to 40	26	1.5	73	3.9
50 to 59	315	18.0	355	19.0
60 to 69	639	36.5	692	37.0
70 to 79	534	30.5	591	31.6
80 and over	236	13.5	159	8.5

This group was given 0.5 cc. containing 1 mg. each of type I and type II of the active substance. Another 1,000 were taken from the old inmates and divided into two groups of 500 each. Five hundred were inoculated with 1.0 cc. containing 2 mg. of each active substance. The 500 who had been used as controls the previous year were again used as controls.

RESULTS

Age Distribution of Immunized and of Control Group.—The age distribution of the inoculated and of the control group is given in table 1. This shows that in the immunized group the age distribution was as follows: 36.5 per cent between the ages of 60 and 69 years, 30.5 per cent between 70 and 79 years, 18 per cent between 50 and 59 years, 13.5 per cent 80 years or over and 1.5 per cent 40 years or below. In the control group the age distribution was 37 per cent between 60 and 69 years, 31.6 per cent between 70 and 79 years, 19 per cent from 50 to 59 years, 8.5 per cent 80 years or over and 3.9 per cent were 40 years or below. It can be seen that both groups had about the same age distribution. For equalizing purposes the general mortality rates from all causes were checked for the immunized and for the control group and found to be essentially the same.

Effect on Incidence and Mortality Among Immunized Group Compared with Control Group.[19]—First Year: In the first year the number of cases of pneumonia among the 1,000 immunized persons was 14, giving an incidence rate of 14 per thousand. The number of deaths in the immunized group was 8, giving a mortality rate of 8 per thousand. Among the 1,120 nonimmunized there were 63 cases of pneumonia, making the incidence 56 per thousand. The number of deaths among the 1,120 controls was 47, making a mortality rate of 42 per thousand (table 2).

TABLE 2.—*Incidence and Mortality Rates Among Inoculated and Noninoculated in the First and in the Second Year of Observation*

	Number of Persons	Number of Cases of Pneumonia	Incidence Rate per Thousand	Number of Deaths	Mortality Rate per Thousand
1937-1938					
Inoculated	1,000	14	14	8	8
Noninoculated	1,120	63	56	47	42
1938-1939					
Inoculated	750	9	12	5	6.6
Noninoculated	750	41	55	20	26.6

TABLE 3.—*Analysis of Incidence of Pneumonia in 1938 and 1939 Among Inoculated and Control Groups*

	No. of Persons	No. of Cases of Pneumonia	Incidence, %	Standard Deviation of Percentage	Difference of Percentage	Standard Deviation of Percentage Difference	Critical Ratio	Certainty
1937-1938								
Inoculated	1,000	14	0.014	±0.004	0.042	±0.009	$\frac{\pm 0.042}{\pm 0.009} = \pm 4.7$	100%
Noninoculated	1,120	63	0.056	±0.008				
1938-1939								
Inoculated	750	9	1.2	±0.004	0.043	±0.00984	$\frac{\pm 0.043}{\pm 0.00984} = \pm 4.38$	100%
Noninoculated	750	41	5.5	±0.009				

Second Year: The second year there were 9 cases of pneumonia among the 750 immunized persons and 41 among the 750 nonimmunized, making the incidence rates 12 per thousand in the immunized group and 55 per thousand in the control group. The number of deaths among the 750 immunized persons was 5, making a mortality rate of 6.6 per thousand. Among the 750 nonimmunized the number of deaths was 20, making a rate of 26.6 per thousand (table 2).

19. A sample analysis of the incidence rates is given in table 3, which shows a critical ratio of 4.7 for the year 1938 and one of 4.38 for the year 1939.

Subdivision of Groups in Second Year: As was mentioned before, in the second year the subjects were divided into two groups. Two hundred and fifty new patients received 1 mg. each of type I and type II active substance, and 500 received 2 mg. each. There were 4 cases of pneumonia with 2 deaths in the group of 250 and 5 cases with 3 deaths

TABLE 4.—*Comparison of Incidence and Mortality Rates of Those Receiving One Inoculation of 1 Mg. and Those Receiving Two Inoculations of 1 Mg. Each of Active Substance (1938-1939)*

	No. of Persons	No. of Inoculations	Amount of Polysaccharide, Mg.	No. of Instances of Pneumonia	Incidence per Thousand	No. of Deaths	Mortality per Thousand
Inoculated							
Group I.....	250	1	1	4	16	2	8
Group II.....	500	2	2	5	10	3	6
Controls							
Group I.....	250	..	..	15	60	8	32
Group II.....	500	..	..	26	52	12	24

TABLE 5.—*Summary of Incidence, Mortality and Case Fatality of Pneumonia During Two Years of Experiments*

	No. of Persons	No. of Instances of Pneumonia	Incidence, %	Standard Deviation of %	Difference of %	Standard Deviation of % Difference	Critical Ratio	Certainty
	Incidence per Thousand Persons							
Inoculated.....	1,750	23	1.4	0.003	4.2	0.0067	$\frac{\pm 0.042}{\pm 0.0067} = \pm 6.3$	100%
Noninoculated.....	1,870	104	5.6	0.006				

	Mortality per Thousand Persons		
	No. of Persons	No. of Deaths	Rate per 1,000
Inoculated.....	1,750	13	7
Noninoculated.....	1,870	67	35.8

	Fatality per Hundred Cases							
	No. of Cases of Pneumonia	No. of Deaths	Case Fatality, %	Standard Deviation of %	Difference of %	Standard Deviation of % Difference	Critical Ratio	Certainty
Inoculated.....	23	13	0.57	0.009	0.07	0.11	$\frac{\pm 0.07}{\pm 0.11} = \pm 0.627$	73%
Noninoculated.....	104	67	0.64	0.048				

in the group of 500. In the smaller control group there were 15 cases of pneumonia with 8 deaths and in the larger control group 26 cases with 12 deaths. As can readily be seen, there was no significant difference in the incidence and mortality rates between those receiving two injections of 1 mg. each and those receiving one injection of 1 mg. (table 4).

Total Period of Observation (table 5).—Among the total 1,750 inoculated persons there were 23 cases of pneumonia, making the rate

13.13 per thousand. Among the total 1,870 controls there were 104 cases, making the rate 55.66 per thousand. There were 13 deaths in the immunized group, making the mortality rate 7.4 per thousand. In the control group the number of deaths was 67, making a mortality rate of 35.8 per thousand.

Effect of Immunization on Case Fatality Rates.—The number of deaths among the 23 inoculated patients with pneumonia was 13, making a case fatality rate of 57 per cent. In the control group the number of deaths among 104 patients with pneumonia was 67, making a case fatality rate of 64 per cent. The statistical analysis of these figures which is given in table 5 shows that the critical ratio is 0.627 and the percentage of certainty is 73. Nevertheless it does not seem justified to draw conclusions from the case fatality rate calculated on such a small number of deaths.

TABLE 6.—*Pneumonia Mortality and Incidence per Thousand Years of Life*

	No. of Persons	Years of Life Exposed	No. of Instances of Pneumonia	Incidence Rate	No. of Deaths	Mortality Rate
Inoculated						
1938	1,000	1,890.4	14	7.4%	8	4.2%
1939	750	708.9	9	12.6%	5	7.0%
Total	1,750	2,599.3	23	8.8% of total	13	5.0% of total
Noninoculated						
1938	1,120	2,092.7	63	30.1%	47	22.4%
1939	750	684.2	41	39.9%	20	29.2%
Total	1,870	2,776.9	104	37.4% of total	67	24.1% of total

Comparison of Incidence and Mortality Rates Observed in Two Years of Experiment With Those of Previous Ten Years.—Since many of the persons who were under observation in these experiments were in the institution for years and represented a cross section of the total, we had a fairly accurate estimate of their previous incidence and mortality rates. If the rates for the past ten years are compared with those of these two years' experiments, it is seen that whereas the average rate of incidence in the previous ten years was 57 per thousand and the mortality rate was 28.3 per thousand, in these two years of observation the incidence rate among the immunized came down to 13.12 per thousand and the mortality rate to 7.4 per thousand.

Incidence and Mortality Rates Considered on Basis of Years of Life Exposed.—The incidence and mortality rates in the previous paragraphs are calculated per thousand. For comparison it seemed worth while to compute the incidence and mortality rates on the basis of a thousand years of life (as explained in the section on the experiments of Felton, Ekwurtzel, Simmons and Dublin). This computation is shown in table 6.

TABLE 7.—*History of Patients with Pneumonia in the Inoculated Group During the Two Years*

Case Number; Sex; Age, Years	No. of Days from Inoculation to Onset of Pneumonia	Type of Organism (Culture of Sputum and Blood)	Physical Diagnosis	Roentgentographic Findings	Blood Count	Duration of Pneumonia, Days	Results	Autopsy Observations or Comments
1 F 70	4	No pneumococci of types I through XXXII	Lobar pneumonia; (LLL; RLL)	Irregular, patchy, confluent clouding of lower left portion of chest and base of right lung suggestive of bronchopneumonic involvement	Hemoglobin, 86% White blood cells, 15,600 Red blood cells, 4,600,000 Polymorphonuclears, 91% Lymphocytes, 9%	12	Recovery	
2 F 46	27	No organism	Lobar pneumonia (RML)	Massive consolidation in right middle lobe area with some clouding at base, perhaps pleural in origin	Hemoglobin, 86% White blood cells, 25,400 Red blood cells, 4,500,000 Polymorphonuclears, 91% Lymphocytes, 9%	10	Recovery	
3 M 78	32	No pneumococci of types I through XXXII; diplococci; streptococci; no tubercle bacilli	Bronchial pneumonia (RML; LLL)	Patchy confluent pneumonic changes in both midlobe regions	Hemoglobin, 59% White blood cells, 7,700 Red blood cells, 3,700,000 Polymorphonuclears, 73% Lymphocytes, 27%	8	Death	
4 F 62	98	Type XIX	Lobar pneumonia (RLL)	Irregular central consolidation at base of right lung; rest of lungs clear	Hemoglobin, 76% White blood cells, 10,700 Red blood cells, 4,100,000 Polymorphonuclears, 75% Lymphocytes, 23%	12	Recovery	
5 M 69	123	No organisms	Bronchial pneumonia (LLL)	.....	Hemoglobin, 90% White blood cells, 9,400 Red blood cells, 4,660,000 Polymorphonuclears, 75% Lymphocytes, 21%	18	Recovery	
6 M 66	132	Type III	Lobar pneumonia (RLL)	Localized patchy consolidation at base of right lung	Hemoglobin, 90% White blood cells, 12,800 Red blood cells, 4,660,000 Polymorphonuclears, 83% Lymphocytes, 10%	12	Recovery	
7 M 79	137	Type III; blood culture positive	Bronchial pneumonia (RML; RLL)	.....	Hemoglobin, 87% White blood cells, 14,600 Red blood cells, 4,400,000 Polymorphonuclears, 76% Lymphocytes, 17%	18	Death	

8 M 58	141	Type III	Lobar pneumonia with pleural effusion (LLL)	Clouding of entire left chest and deviation of heart to right, indicating pleural effusion	Hemoglobin, 90% White blood cells, 29,300 Red blood cells, 4,000,000 Polymorphonuclears, 82% Lymphocytes, 14%	2	Death	
9 F 69	176	Cultures not made	Bronchial pneumonia (RML)	No roentgen examination	Hemoglobin, 80% White blood cells, 13,600 Red blood cells, 4,370,000 Polymorphonuclears, 75% Lymphocytes, 24%	4	Death	Autopsy: pulmonary congestion; lobar pneumonia; cardiac hypertrophy; dilatation of heart; right coronary sclerosis; myocardial fibrosis; hypertensive kidney; atrophy of uterus and ovaries; decrease in size of parenchymatous organs
10 M 85	191	No organisms	Lobar pneumonia (RLL; LLL)	Irregular, patchy confluent pneumonia; changes in both lower lobes	Hemoglobin, 90% White blood cells,28,600 Red blood cells, 4,800,000 Polymorphonuclears, 90% Lymphocytes, 10%	2	Death	
11 F 68	225	Type II; blood culture positive	Bronchial pneumonia (LLL)	No roentgen examination	Hemoglobin, 83% White blood cells, 9,800 Red blood cells, 4,000,000 Polymorphonuclears, 80% Lymphocytes, 19%	6	Death	Autopsy: bronchopneumonia; hypertrophy of heart; congestion and fatty degeneration of liver; arteriosclerosis, left kidney; congenital hypoplasia, right kidney
12 M 75	236	Cultures not made	Bronchial pneumonia (RLL)	At base of right lung, irregular patchy pneumonia exudate; dilatation of arch and descending aorta; a soft basic shadow projecting laterally from left cardiac border	Hemoglobin, 80% White blood cells, 12,800 Red blood cells, 4,220,000 Polymorphonuclears, 85% Lymphocytes, 13%	7	Recovery	
13 M 79	258	Long chained streptococci	Bronchial pneumonia	One small calcified area at base of right lung and small area in left hilus indicating pulmonary tuberculous infection; no pulmonary infiltration or consolidation	Hemoglobin, 88% White blood cells, 9,800 Red blood cells, 4,510,000 Polymorphonuclears, 81% Lymphocytes, 19%	12	Death	Autopsy: lobular pneumonia; cardiac hypertrophy; coronary sclerosis; horseshoe kidney
14 M 48	292	No tubercle bacilli; no pneumococci of types I through XIV	Bronchial pneumonia (RML; RLL)	.....	Hemoglobin, 90% White blood cells, 8,600 Red blood cells, 4,700,000 Polymorphonuclears, 60% Lymphocytes, 29%	6	Death, 10th day	
15 F 50	307	Type I; blood culture negative	Bronchial pneumonia (RLL)	Central consolidation occupying inner half of lower lobe of right lung; rest of lungs clear	Hemoglobin, 82% White blood cells, 15,800 Red blood cells, 4,400,000 Polymorphonuclears, 85% Lymphocytes, 13%	10	Recovery	
16 M 70	324	Type III; blood culture positive	Lobar pneumonia (LML)	Opacity in right side of chest between second and fifth ribs; irregular exudative nodules in first intercostal space	Hemoglobin, 90% White blood cells, 14,700 Red blood cells, 4,500,000 Polymorphonuclears, 89% Lymphocytes, 11%	8	Death	

TABLE 7.—*History of Patients with Pneumonia in the Inoculated Group During the Two Years—Continued*

Case Number; Sex; Age, Years	No. of Days from Inoculation to Onset of Pneumonia	Type of Organism (Culture of Sputum and Blood)	Physical Diagnosis	Roentgentographic Findings	Blood Count	Duration of Pneumonia, Days	Results	Autopsy Observations or Comments
17 M 81	331	No organism	Bronchial pneumonia (LLL)	No pulmonary consolidation delineated	Hemoglobin, 72% White blood cells, 8,600 Red blood cells, 3,800,000 Polymorphonuclears, 79% Lymphocytes, 18%	5	Death	Autopsy: hypostatic pneumonia
18 M 41	342	No pneumococci of types I through XXXII; streptococci in small and large chains	Bronchial pneumonia (LLL)	.....	Hemoglobin, 90% White blood cells, 10,200 Red blood cells, 4,520,000 Lymphocytes, 18%	11	Recovery	
19 M 67	353	No organisms	Bronchial pneumonia (RLL; LLL)	.....	Hemoglobin, 90% White blood cells, 9,200 Red blood cells, 4,520,000 Polymorphonuclears, 57% Lymphocytes, 40%	14	Death, 18th day	Autopsy: confluent lobular pneumonia (RLL, RUL, possibly LLL)
20 M 85	361	No organisms	Bronchial pneumonia (RLL)	Irregular patchy consolidation in lower lobe of right lung; rest of lung clear	Hemoglobin, 90% White blood cells, 15,800 Red blood cells, 4,600,000 Polymorphonuclears, 90% Lymphocytes, 9%	6	Death	Autopsy: no effusion in pleural cavities; many pleural adhesions on the right; pulmonary fibrosis in both lungs, more to right; lobular pneumonia (RLL); showed considerable arteriosclerotic changes in large pulmonary vessel
21 M 75	364	Type III	Bronchial pneumonia (RLL)	Irregular confluent consolidation at base of right lung; rest of lungs clear	Hemoglobin, 95% White blood cells, 11,300 Red blood cells, 4,800,000 Polymorphonuclears, 84% Lymphocytes, 10%	16	Death, 12th day	Autopsy: pulmonary edema; lobular pneumonia (RLL)
22 F 73	378	Type IV	Lobar pneumonia (LVL)	Marked clouding on the right side extending from apex to fourth righ anteriorly, suggesting a pneumococcic consolidation; conclusion, lobar pneumonia	Hemoglobin, 85% White blood cells, 16,400 Red blood cells, 4,530,000 Polymorphonuclears, 88% Lymphocytes, 11%	9	Recovery	
23 M 78	489	Type I; blood culture negative	Bronchial pneumonia (RLL)	No pulmonary infiltration or consolidation; numerous healed rib fractures		10	Recovery	

The subjects in the first year's experiment were observed for two years, with the exception of those who died from causes other than pneumonia and the fraction who were discharged and could not be followed up. Persons in the second year's experiment were observed for one year, with the same exceptions.

For the first group the value representing "years of life exposed" among the inoculated was 1,890 and among the controls 2,092. For the second group the number of "years of life exposed" among the inoculated was 708 and among the controls 684. Both groups being considered together, the incidence rate for the inoculated was 6.8 per thousand years of life and for the control group 37.4. From this it can be seen that the incidence rate for the noninoculated group was 4.25 times that for the inoculated.

The mortality rate calculated on the basis of a thousand years of life for the inoculated group was 5 and for the noninoculated 24.1, the rate for the noninoculated being almost 5 times higher than that for the inoculated group.

General and Local Reactions.—Among all the immunized (1,750 persons) there were 86 reactions. Of these 51 had local reactions, showing redness of the skin around the point of injection, and 31 redness extending to the upper part of the arm. Four patients had, aside from the local reactions, general malaise and elevation of temperature to between 100 and 101.2 F., which subsided in all cases within twenty-four hours. The milder local reactions disappeared in ten to sixteen hours. No serious local or general reaction was noted.

Clinical Analysis of Cases of Pneumonia Among Inoculated Persons (Table 7).—There were 7 patients with lobar pneumonia, with 3 deaths, and 15 with bronchial pneumonia, with 10 deaths, in the inoculated group. At postmortem examination, 1 patient was found to have hypostatic pneumonia.

The type distribution in the two groups was as follows: In the inoculated group, 2 patients with type I, 1 with type II, 4 with type III, 1 with type IV and 1 with type XIX. In 2 cases nontypable diplococci and in 4 cases, long chains of streptococci were found, and the findings in 8 cases were bacteriologically irrelevant. In the control group there were 6 patients with type I, 3 with type II, 13 with type III, 2 with type IV, 3 with type VI, 3 with type VII, 1 with type IX, 1 with type XIV and 1 with type XIX. Nontypable pneumococci were found in cultures of material from 21 cases, long-chained streptococci in 22 cases and Koch's bacilli in 1 case, and in 25 cases the findings were bacteriologically irrelevant.

An analysis of the white blood cell counts (table 8) shows that there were 8 patients with between 10,000 and 15,000 leukocytes, 7 with less than 10,000, 4 with between 15,000 and 20,000 and 3 with between 25,000 and 30,000.

The average duration of the disease was nine days. Pleural effusion was found in 2 cases. More than one lobe was involved in 4 cases. Bacteremia was found in 3 cases.

TABLE 8.—*Summary of White Blood Counts of Patients with Pneumonia in Inoculated Group* *

White Blood Count	No. of Cases	No. of Recoveries	No. of Deaths
Under 10,000	7	1	6
10,000-14,900	8	4	4
15,000-19,900	4	3	1
20,000-24,900	0	0	0
25,000-30,000	3	1	2

* Case 23 is unaccounted for.

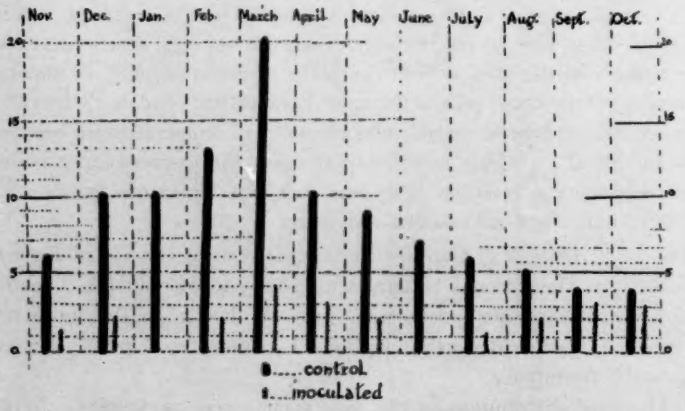

Chart showing month by month incidence of pneumonia among inoculated and control groups for two years.

It may be concluded from the clinical analysis of the cases of pneumonia among the inoculated group that the disease in that group did not show any remarkable feature (as expressed by the course or duration of the disease, the complications or the case fatality rates) which would make it different from the usual clinical picture of old age pneumonia.

Duration of Immunity.—The chart shows that the highest number of instances of pneumonia among the controls occurred, as is common on the eastern seaboard, in the month of March. There was an almost regular increase from November to March and a fairly regular decrease

from then on to the end of October. The same chart shows a different picture for the inoculated group. The incidence was almost even in November, January and February, with a relatively smaller seasonal increase in March. From here it was practically on an even level until August, when the height of the column reached about the same level as in March. In September and October it came down somewhat but it was relatively still higher than that for the noninoculated group. An analysis of the figures contained in the two tables (table 7, column 4; table 9) also shows that there were relatively more cases in the second than in the first half of the year. Although the number of cases was too small to warrant conclusions, there is an indication that the effect of the immunity was less pronounced in the last quarter than in the first three quarters of the year after immunization.

Isolated Epidemic in Two Wards.—In one of the buildings (wards 51 and 52) an isolated epidemic of pneumonia occurred in the late spring

TABLE 9.—*Interval Between Inoculation and Onset of Pneumonia*

Days From Inoculation to Onset of Pneumomnia	No. of Cases
0- 60	3
61-120	1
121-180	5
181-240	3
241-300	2
301-360	5
361-420	3
421-480	0
481-540	1

of 1939. There were 22 patients with pneumonia in a building where 154 people lived. Of these 154 persons 63 were inoculated in November 1938. There were among them 5 patients with pneumonia, 2 with lobar and 3 with bronchial pneumonia, and 2 of the patients died. Among the controls there were 17 with pneumonia, 8 with lobar and 9 with bronchial pneumonia; of these, 10 died. The type distribution in this epidemic was as follows: Among the inoculated, the infection in 1 patient was due to pneumococci of type II and in 1 to long-chained streptococci and in the rest the organisms could not be typed. Among the controls the organisms were: in 2 pneumococci of type I, in 1 type II, in 1 type III, in 1 type XIX and in 3 pneumococci which could not be typed. Cultures in 2 cases showed streptococci, and in the rest the findings had no bacteriologic relevance. As can be seen, the data of this epidemic seem to confirm the findings of the main body of the experiments.

COMMENT

It is fairly well established that vaccines of killed or attenuated pneumococci have a prophylactic value. Not only animal experiments

but large scale inoculations done on human beings seem to substantiate this view.[20]

The antigen used in these experiments is made from a fraction of the pneumococcus. It is a polysaccharide, containing nitrogen as well as carbohydrates and having no acetyl radical. Its chemical structure is not yet entirely known. No doubt the different preparations, sometimes even the different batches, are chemically not identical. But all contain dextrose and nitrogen. Felton and Kauffmann [21] stated the belief that proteins as well as lipoids and enzymes are primarily absent from the antigen. Goebel [22] and Heidelberger, Kendall and Scherp,[8] assumed on the basis of analysis that it contains uronic acid and nitrogen. This is apparently near the final chemical structure, since with an artificial antigen chemically similar to the polysaccharides (azobenzylglycoside of cellobiuronic acid) Goebel succeeded, in experiments on mice, in conferring protection against type II, III and VIII pneumococci. In this way it was shown that not only the pneumococcus polysaccharides but their synthetic counterparts are capable of multiple antigenic action.

The data of the experiments reported here seem to substantiate these findings as far as the action of pneumococcus polysaccharides is concerned. The incidence and mortality rates of pneumonia were reduced not only for the types from which these substances were isolated but also for other types. It is true that the number of persons observed was relatively small (in the immunized and control groups together being 3,620), and since the period of observation was only two years, further observation is necessary to corroborate the data presented in this paper.

It seems obvious, however, that a group like the one used in these experiments is particularly suited for immunizations against pneumonia. As already pointed out, the high incidence, mortality and case fatality rates and the frequency of repeated attacks of pneumonia make active immunization among them a practical problem. Furthermore, since postoperative pneumonia is a dreaded complication in this age group, immunization with polysaccharide (if its prophylactic value can be definitely established) seems to be a procedure promising considerable reduction in the occurrence of pneumonia after surgical procedures.

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SUMMARY AND CONCLUSIONS

A report on active immunization with pneumococcic capsular polysaccharide against old age pneumonia in 1,750 subjects in a study conducted during the past two years is given.

The data on the incidence, mortality and case fatality rates of the immunized are compared with those of 1,870 control patients.

The following observations were made:

1. While the incidence and mortality rates seem to be reduced among the inoculated, there is no appreciable effect on the case fatality rates.

2. The course of pneumonia in the immunized group (as expressed by the length of the disease and the frequency of complications) was apparently not influenced by the inoculations.

3. The prophylactic value seemed more pronounced in the first three quarters than in the last quarter of the year after immunization.

FURTHER STUDIES ON HUMAN CARDIAC AND VOLUNTARY MUSCLE

POSSIBLE IMPLICATIONS OF CHANGES IN THE CREATINE, PHOSPHORUS AND POTASSIUM CONTENT, WITH SPECIAL REFERENCE TO HEART DISEASE

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A number of studies have shown that the creatine concentration of cardiac and voluntary muscle is decreased in certain pathologic conditions. This work has been summarized in an earlier paper from this laboratory.[1] The fundamental cause of the decrease in concentration of creatine in heart failure and other pathologic conditions has not been elucidated. However, certain facts are known that have an important bearing on this subject. Creatine is present in muscle largely in the form of phosphocreatine.[2] That the exact amount present in this form cannot be determined by analysis is due to its rapid breakdown during manipulation of the sample. The best analyses suggest that about 75 to 80 per cent of the creatine present in voluntary muscle is in the form of phosphocreatine while in cardiac muscle the relative amount present in this form is much lower. Creatine can be readily washed out of

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The chemical data in this paper are taken from a dissertation submitted by George H. Mangun to the Graduate School of Western Reserve University, June 1938, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

1. Linegar, C. R.; Frost, T. T., and Myers, V. C.: Variation in Creatine Content of Human Cardiac and Voluntary Muscle at Autopsy, Arch. Int. Med. **61**:430 (March) 1938.

2. Fiske, C. H., and Subbarow, Y.: Phosphocreatine, J. Biol. Chem. **81**:629, 1929.

cardiac muscle by perfusion.[3] In skeletal muscle the rate at which creatine [4] diffuses out has been shown to be greater in fatigued than in resting muscle, probably because of a greater dissociation of phosphocreatine. That the retention of creatine by muscles is not due to impermeability of the cells is suggested by the fact that administered creatine can be taken up by muscle.[5] It has been shown that the administration of creatine [6] causes a decrease in the amount of phosphorus in the urine, evidence that this creatine is actually stored in the tissues as phosphocreatine. This evidence, together with a large body of other facts, suggests that variations in the creatine content of cardiac and voluntary muscle are, under conditions of equilibrium, reflections of variations in the metabolism of phosphocreatine.

Phosphocreatine is strongly acid in character and at the p_H commonly assigned to the interior of muscle cells would be largely present in the dibasic form. Since potassium is the chief base of the cells, it appears logical to assume that phosphocreatine exists in the living cells as dipotassium phosphocreatine.[7] One might, therefore, anticipate a parallel decrease in creatine, phosphorus and potassium if the loss of creatine is attributed to a breakdown of phosphocreatine or failure of its resynthesis. Calhoun, Cullen, Clark and Harrison [8] have shown that the total phosphorus and potassium are decreased in the hearts of persons dying of congestive heart failure, an observation in accord with the aforementioned hypothesis.

In the present study, creatine, phosphorus and potassium determinations were carried out on muscle from the left and right ventricles of the heart and on skeletal muscle (pectoralis major) to establish what relation, if any, existed among the losses of these muscle constituents.

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METHOD

Samples were obtained from the left and right ventricles and the pectoralis major muscle of human beings during autopsy, according to the method described by Seecof, Linegar and Myers.[9] The analyses were carried out on a single sample as described in another paper,[10] dealing with normal tissue, the creatine being determined as described in earlier papers in this series,[11] the phosphorus by the method of Fiske and Subbarow[12] and the potassium by the permanganate titration method of Kramer and Tisdall.[13] Care was taken to avoid possible postmortem changes, and with 4 exceptions all samples were taken within twenty-four hours post mortem.

Determinations were carried out on muscle from the left and right ventricles of 95 human beings. For 61 of them, analyses were also made of voluntary muscle (pectoralis major). Thirteen of the 61 were victims of sudden death, and the samples of their muscles were obtained at the Cuyahoga Country Morgue; the data on these subjects were discussed in a separate paper.[10] The remaining subjects have been classified in groups according to the major diagnoses or included in the miscellaneous group.

RESULTS

The average results obtained for the 13 normal persons and the 82 with a pathologic condition are summarized in table 1. The difference between the left and the right ventricle was very nearly the same in the normal subjects and in those with a pathologic condition. The probable source of this difference between the two ventricles will be discussed in more detail in a later section, but at this point it is well to establish the normal relationships. The average difference between the left and the right ventricle was 22.1, 20.3 and 23.2 per cent for creatine, phosphorus and potassium respectively, the left ventricle being used as the standard of reference. The finding of essentially the same percentage of difference between the left and the right ventricle for each constituent suggests that muscle dilution may be the principal factor responsible for the differences between the two ventricles.

In all but 2 instances, the concentrations were higher in the left ventricle than in the right. Seecof, Linegar and Myers[9] found a higher concentration of creatine in the left ventricle in every instance in a series of 108 human hearts, except in 1 newborn infant.

Myocardial Insufficiency.—Included in this group were only those patients whose condition was clinically diagnosed as myocardial insuf-

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ficiency and who showed evidence of progressive decompensation with hyperemia of the internal organs and edema of the extremities. Patients with less distinct signs of myocardial insufficiency were placed with the group having miscellaneous cardiac involvements. All patients with

TABLE 1.—*Summary of Observations on Creatine, Phosphorus and Potassium Content of Cardiac and Voluntary Muscle in 13 Normal and 82 Diseased Persons (Mg./100 Gm. of Wet Tissue)*

		Creatine		Phosphorus		Potassium	
		Normal	Diseased	Normal	Diseased	Normal	Diseased
Left ventricle.....	Average	203	199	194	187	285	267
	Maximum	218	330	204	235	305	332
	Minimum	186	99	180	108	251	170
Right ventricle.....	Average	165	155	160	149	219	205
	Maximum	184	238	187	227	242	275
	Minimum	154	70	140	105	189	114
Pectoralis major *...	Average	443	405	201	192	328	314
	Maximum	462	538	216	245	346	415
	Minimum	442	212	195	115	311	169

* Thirteen normal persons and 48 with some pathologic condition.

TABLE 2.—*Myocardial Insufficiency (Values Calculated per Hundred Grams of Fresh Tissue)*

					Left Ventricle			Right Ventricle			Voluntary Muscle		
Number	Age	Sex	Hours Post Mortem	Heart Weight, Gm.	Creatine, Mg.	Phosphorus, Mg.	Potassium, Mg.	Creatine, Mg.	Phosphorus, Mg.	Potassium, Mg.	Creatine, Mg.	Phosphorus, Mg.	Potassium, Mg.
72.....	42	F	6	350	170	177	241	121	141	190	371	182	276
60.....	67	F	4	400	169	173	245	121	135	182	344	183	264
43.....	60	F	55	425	167	175	223	131	152	164	321	167	245
31.....	65	F	2	375	165	169	260	113	144	196	...	...	...
19.....	68	M	7	600	161	171	185	110	132	152	...	...	...
37.....	39	M	..	675	159	197	206	116	125	153	338	138	265
67.....	52	M	12	475	155	180	233	137	144	189	343	190	256
6.....	53	M	9	550	154	108	225	147	107	192	...	...	...
75.....	58	M	2	525	148	170	236	126	139	189	357	190	247
15.....	58	M	6	550	145	152	244	135	141	134	...	...	...
36.....	30	F	..	825	143	172	222	117	114	173	...	...	...
11.....	59	M	54	525	143	166	293	91	105	195	...	...	...
42.....	39	M	5	750	131	177	174	136	187	184	278	134	336
39.....	53	M	9	800	128	190	251	70	135	179	333	194	285
4.....	56	M	78	575	126	147	170	88	117	145	...	...	...
45.....	42	M	5	600	120	180	290	72	157	201	305	195	350
41.....	43	M	4	450	99	171	244	78	138	218	...	...	...
Average.....					146	169	232	112	135	178	349	177	287

clinical proof of nitrogen retention were grouped together as having uremia with heart failure. No patients were included here who showed anatomic evidence of severe renal disease, even though there was no clinical proof to substantiate the anatomic findings. Such patients were included in the group with miscellaneous cardiac involvements.

It becomes evident from an inspection of table 2 that the concentrations of creatine, phosphorus and potassium were decreased in both

ventricles of the heart and in the voluntary muscle. In every case the creatine content of the left ventricle was below the normal level. The concentration of phosphorus was likewise lowered, but to a lesser degree, in all but 2 cases. The potassium decreased in all but 2 cases, the average percentage of decrease being somewhat more than that of phosphorus and less than that of creatine. Similar findings were recorded for the right ventricle. In every case the creatine concentration of the voluntary muscle was below the normal level, that of phosphorus and that of potassium were more variable, but the average values were definitely lowered. After the changes were analyzed more closely, it was believed to be more to the point to consider that portion of each constituent lost, rather than changes occurring in the total amount present in normal muscle. This assumption arose from the fact that

TABLE 3.—*Molecular Relationships Among the Losses of Creatine, Phosphorus and Potassium (Millimols per Kilogram of Wet Tissue)*

	Left Ventricle			Right Ventricle		
	Creatine	Phosphorus	Potassium	Creatine	Phosphorus	Potassium
Average of 91 patients..	15.1	60.5	68.8	11.8	48.3	54.2
Congestive heart failure	11.1	54.5	59.4	8.5	43.5	46.5
Decrease, millimols	4.0	6.0	9.4	3.3	4.8	7.7
Decrease, percentage	26.6	10.1	13.7	28.0	10.0	14.2
Mols required for loss of dipotassium phosphocreatine	4.0	4.0	8.0	3.3	3.3	6.6
Additional P and K lost	....	2.0	1.4	....	1.8	1.1

all of the phosphorus and potassium present is not directly concerned with creatine. Therefore, if the changes observed were primarily due to variations in the creatine-phosphocreatine mechanism, only that part of the phosphorus and potassium directly associated with the creatine might be expected to vary. On the other hand, if the observed decreases were due chiefly to dilution of the active muscle tissue with water, fat or connective tissue, one might anticipate that the whole of the creatine, phosphorus and potassium would be affected. The net result would be that a decrease of 10 per cent, for example, in the creatine would be accompanied by a decrease of 10 per cent in the phosphorus and potassium.

Table 3 shows the changes that occurred in this group in terms of percentage decrease in the three constituents and in terms of loss in millimols per kilogram. From these data it is apparent that tissue dilution was not the important factor involved, since there was no correlation between the percentage decreases of the three constituents in either ventricle.

If one assumed a loss of the constituents of dipotassium phosphocreatine, using the loss of 4 mols of creatine as the standard of reference, one would expect a loss of 4 mols of phosphorus and 8 mols of potassium. The actual loss of the latter two exceeded that of creatine. This fact is suggestive of a loss of adenylpyrophosphate, in accordance with the findings of Burns and Cruickshank [3a] and others that this compound may break down under a variety of experimental conditions.

TABLE 4.—*Miscellaneous Cardiac Involvements (Values Calculated per Hundred Grams of Fresh Tissue)*

					Left Ventricle			Right Ventricle			Voluntary Muscle		
Number	Age	Sex	Hours Post Mortem	Heart Weight, Gm.	Creatine, Mg.	Phosphorus, Mg.	Potassium, Mg.	Creatine, Mg.	Phosphorus, Mg.	Potassium, Mg.	Creatine, Mg.	Phosphorus, Mg.	Potassium, Mg.
2.....	55	M	13	625	294	163	240	132	122	210	...	...	...
53.....	55	F	10	325	253	197	286	192	154	155	343	192	284
55.....	77	F	2	450	252	168	282	198	143	190	353	156	257
51.....	32	M	3	750	236	175	284	194	143	184	405	182	304
1.....	35	F	1	325	233	173	256	195	158	230	...	...	...
54.....	53	M	4	770	213	181	285	169	152	212	339	178	288
40.....	56	M	7	360	206	199	292	189	134	119	...	...	...
59.....	42	F	4	375	202	197	264	148	147	207	421	198	314
23.....	67	M	9	475	195	210	307	160	202	243	...	...	...
38.....	50	M	6	485	194	186	260	119	119	171	...	...	...
32.....	49	M	9	400	192	197	250	97	93	134	480	206	270
56.....	86	F	2	175	191	182	273	136	141	191	...	...	...
13.....	45	M	2	375	190	189	332	168	142	236	...	...	...
16.....	73	F	6	150	185	106	187	119	114	137	...	...	...
66.....	58	F	9	500	186	188	251	142	148	197	347	182	276
63.....	62	M	7	525	177	178	254	139	147	193	394	191	307
44.....	58	M	5	425	176	197	292	105	155	165	388	226	322
26.....	55	M	16	600	173	194	251	154	180	239	...	...	...
69.....	61	F	7	475	165	179	234	126	137	180	374	186	294
14.....	36	M	4	375	164	162	250	147	148	223	...	...	...
28.....	64	F	3	350	163	210	200	119	153	127	415	184	197
50.....	73	M	5	500	163	161	217	125	136	100	212*	113*	109*
22.....	62	F	11	650	157	212	285	144	182	275	...	...	...
34.....	52	F	3	500	141	185	229	113	138	197	...	...	...
Average.....					195	185	261	147	145	190	384	188	283

* This sample of muscle contained a large amount of fat; the values were not used in calculating the averages.

It is improbable that any extensive changes occur in compounds of phosphorus other than phosphocreatine and adenylpyrophosphate. Phosphate esters are present in too small quantities to account for the phosphorus decreases observed in these human hearts. Inorganic phosphorus would be more likely to increase than to decrease because of the liberation of phosphorus from phosphocreatine and adenylpyrophosphate. As the heart is stimulated to greater work and as its nutritional condition becomes poorer, fatigue and anoxemia may become progressively greater until the heart is no longer able to resynthesize phosphocreatine and adenylpyrophosphate as fast as they are broken down.

Miscellaneous Cardiac Involvements.—This group included all patients who showed significant changes in the heart but did not clinically manifest appreciable cardiac insufficiency.

It is worthy of note that of the first 6 patients in this group, 5 showed anatomic evidences of renal involvement (patients 2, 53, 55, 51 and 54). However, in the absence of clinical evidence of retention, they were excluded from that classification. The condition in patient 54 was also clinically diagnosed as cardiac failure, but the patient was excluded from this classification because of the possible nitrogen retention.

In 4 instances (patients 2, 40, 38 and 44) of right-sided heart failure (cor pulmonale), the left ventricle averaged 217, 186 and 271 mg. of creatine, phosphorus and potassium and the right ventricle 136, 132 and 166 mg. respectively in each 100 Gm. On the basis of the chemical data, it is apparent that when the left ventricle fails, the right ventricle also

TABLE 5.—*Uremia Without Heart Failure (Values Calculated per Hundred Grams of Fresh Tissue)*

					Left Ventricle			Right Ventricle			Voluntary Muscle		
Number	Age	Sex	Hours Post Mortem	Heart Weight, Gm.	Creatine, Mg.	Phosphorus, Mg.	Potassium, Mg.	Creatine, Mg.	Phosphorus, Mg.	Potassium, Mg.	Creatine, Mg.	Phosphorus, Mg.	Potassium, Mg.
83	37	M	3	340	230	218	310	165	143	...	502	245	367
47	37	M	4	400	272	172	280	220	165	194	431	244	390
52	43	M	6	275	266	213	317	238	176	241	487	202	352
35	50	F	12	520	205	217	331	134	144	197	4[illegible]	216	314
48	56	M	3	350	248	195	252	207	126	204	4[illegible]	208	415
70	54	F	11	425	247	211	302	186	164	241	408	221	337
80	78	M	2	450	246	184	284	135	128	198	588	195	338
78	60	M	10	275	215	214	257	185	153	160	438	192	329
12	4½	M	4	125	214	188	271	174	165	204	...	...	...
49	47	M	2	375	212	189	266	202	181	215	495	187	295
71	40	M	5	350	194	190	271	121	128	181	418	207	331
Average					246	199	286	182	153	205	461	212	347

fails, but the right ventricle may show chemical evidence of failure without similar changes occurring in the left ventricle, as is evident from this small series of 4 cases.

In 1 case of coronary thrombosis (patient 13), in which the sample was taken from an area that was not infarcted, the values for all three constituents were within the normal range. In patient 56 with brown atrophy of the heart, the values were slightly below normal with the exception of that for potassium in the left ventricle.

Uremia without Heart Failure.—Table 5 lists the patients who were classified as having uremia without heart failure. Nearly all of these patients' hearts were diseased or hypertrophic to some extent, but the clinical signs of heart failure were either absent or moderate. Those patients with cardiac decompensation have been grouped together as patients with uremia with heart failure. This classification was based on the earlier findings of Linegar, Frost and Myers.[1]

The creatine content of both ventricles and that of the voluntary muscle were distinctly higher than in the 13 normal hearts. In this group the creatine content of the left ventricle was higher than in any of the normal hearts in 7 of 11 patients; that of the right ventricle was above normal range in 7 patients, and that of voluntary muscle was above normal range in 5 of 10 patients.

Although the average phosphorus values for both ventricles were well within the normal range, it should be noted that 5 of the values for the left ventricle were definitely above the normal limits, while those for the right ventricle were lower and more variable.

The potassium content of the three tissues corresponded closely with that of phosphorus, although there were some individual variations. The average potassium content of the left ventricle was in the upper normal range, and that of the right ventricle was somewhat lower. The potassium content of voluntary muscle appeared to be definitely higher than the normal value. Four of the potassium values were above the normal limits, and four were near the upper normal level.

The most outstanding fact about this group was the relatively high values for voluntary muscle. In all other pathologic conditions that we encountered in this series, the average values were well below the average of the normal group. This was in marked contrast to the situation in cardiac muscle, in which creatine, phosphorus and potassium were undiminished unless cardiac disease was evident and often appeared to be augmented. This difference in the behavior of the two types of muscle may be of considerable physiologic importance. By liberation of these substances from muscle tissue in other parts of the body, the functional capacity of the heart may conceivably be increased during emergencies.

Uremia with Heart Failure.—In this group of patients, the results were much more variable than in the group of those who were classified as having uremia without heart failure. In general, the values for all three constituents were essentially normal for the group, with perhaps a slight elevation in the creatine content and the potassium content of voluntary muscle. One can only say that in cases of uremia with heart failure, high, low or normal values may be encountered, in contrast to the consistently high values in cases of uremia without heart failure.

A comparison of the findings for patients with uremia alone and for patients with heart failure alone leads to the conclusion that here there are two factors with opposite influence on the level of creatine, phosphorus and potassium in cardiac muscle.

Presumably the predominant factor determines the net result, but our present knowledge of the factors involved does not permit any quantitative evalution of them. It may be suggested that in retention, creatine, phosphorus and potassium may be retained in excessive amounts

in voluntary and cardiac muscle only when the nutritional condition, and possibly other unknown factors, is favorable for such a storage. This further suggests that the retention by the muscles of creatine, and also of phosphorus and potassium, is dependent on such a process as the formation of phosphocreatine. In the case of phosphorus and potassium there is the additional possibility that other organic phosphorus compounds may also vary. That such variations do occur is borne out by the lack of correlation in many patients between the values for creatine and those for phosphorus and potassium.

It is quite conceivable that the retention of creatinine (creatinine ⇄ creatine), and also of phosphorus and potassium, in advanced renal disease may have some effect on the course of heart failure. By

TABLE 6.—*Uremia with Heart Failure (Values Calculated per Hundred Grams of Fresh Tissue)*

					Left Ventricle			Right Ventricle			Voluntary Muscle		
Number	Age	Sex	Hours Post Mortem	Heart Weight, Gm.	Creatine, Mg.	Phosphorus, Mg.	Potassium, Mg.	Creatine, Mg.	Phosphorus, Mg.	Potassium, Mg.	Creatine, Mg.	Phosphorus, Mg.	Potassium, Mg.
80.....	41	M	12	600	246	202	282	186	151	...	445	183	276
27.....	57	M	4	575	240	209	301	216	208	272	...	...	...
29.....	49	M	3	625	242	235	295	232	172	248	450	220	286
58.....	33	M	12	425	227	183	275	182	125	226	456	214	400
57.....	49	M	10	500	220	173	305	173	161	242	393	160	359
68.....	47	F	6	550	196	184	258	147	142	191	400	190	311
46.....	60	F	8	300	192	158	236	172	130	186	386	190	401
76.....	56	M	10	600	185	202	231	144	147	...	507	219	335
33.....	66	M	3	550	181	178	218	156	144	189	488	200	362
Average.....					214	192	267	179	153	222	441	199	341

reason of their retention in the blood it is possible that, since they are available in greater amounts than normal, they would promote the resynthesis of phosphocreatine and thus ward off, to some extent at least, the depletion of phosphocreatine and its constituents which ordinarily occurs in the failing heart. At any rate the values for creatine, phosphorus and potassium tend to be definitely higher in patients with uremia without heart failure than in patients with uremia with heart failure, and this is particularly evident in the left ventricle.

Miscellaneous Pathologic Conditions.—Data on patients whose conditions did not fall into one of the major groups are shown in table 7. The left and right ventricles of patients in this group are notable in that the concentrations of creatine, phosphorus and potassium were almost identical or slightly higher than those of the 13 normal persons, while in voluntary muscle the values were appreciably less.

It will be observed that the potassium content of the right ventricle was higher in this group than in the normal group, 231 mg. as compared

with 219 mg. This difference was due probably to a loss of potassium that occurred in some of the normal hearts during the time between autopsy and trimming of the sample. The value of 231 mg. agrees fairly well with the observations of Wilkins and Cullen [14] on the right ventricle.

A comparison of the variability of the values in the group of normal persons and in the group of patients with miscellaneous pathologic conditions reveals a distinct contrast. In the former group the difference between the highest and lowest creatine concentrations of the left ventricle was 32 mg., as compared with 100 mg. in the latter group;

TABLE 7.—*Miscellaneous Pathologic Conditions (Values Calculated per Hundred Grams of Fresh Tissue)*

					Left Ventricle			Right Ventricle			Voluntary Muscle		
Number	Age	Sex	Hours Post Mortem	Heart Weight, Gm.	Creatine, Mg.	Phosphorus, Mg.	Potassium, Mg.	Creatine, Mg.	Phosphorus, Mg.	Potassium, Mg.	Creatine, Mg.	Phosphorus, Mg.	Potassium, Mg.
21	69	M	4	275	270	188	322	178	145	216	...	...	...
82	43	M	6	225	247	202	261	159	137	...	369	186	280
3	18	F	5	150	244	202	295	188	164	240	...	...	...
18	67	F	4	275	238	203	322	195	158	243	...	...	...
25	37	M	50	300	238	180	320	156	139	235	...	...	...
8	58	F	7	225	236	178	275	184	158	232	...	...	...
9	54	M	14	250	232	197	283	194	172	264	...	...	...
61	65	M	5	300	221	197	286	164	159	219	440	204	347
17	50	M	12	350	220	200	324	212	164	260	...	...	...
5	64	M	2	250	216	175	285	148	158	240	...	...	...
62	47	M	4	325	216	192	286	120	141	194	358	172	271
20	20	F	2	200	206	211	314	169	175	264	...	...	...
24	44	F	10	230	205	220	298	220	227	264	...	...	...
74	..	M	14	...	205	192	261	164	157	198	424	204	340
10	50	F	9	210	205	196	310	156	172	224	...	...	...
81	49	M	5	400	204	214	288	125	146	...	376	190	288
64	47	M	3	325	193	190	269	147	153	201	382	196	297
73	..	F	10	...	191	187	255	151	154	197	415	199	299
84	36	M	3	280	186	191	296	153	154	...	431	194	307
65	39	F	14	350	181	186	243	134	145	194	399	187	305
7	1	M	5	50	170	218	301	154	176	271	...	...	...
Average					218	197	286	166	160	231	399	192	303

the phosphorus varied 24 mg. in the former and 45 mg. in the latter, and the potassium varied 54 mg. in the former and 118 mg. in the latter. Similar variations occurred in the right ventricle and in voluntary muscle. The greater degree of variations in the latter group indicated that there were appreciable changes in this group well outside the limits of experimental error.

It would, of course, be of advantage to subdivide the group of patients with miscellaneous conditions further, if by so doing one could differentiate those patients with high values and those with low

14. Wilkins, W. E., and Cullen, G. E.: Electrolytes in Human Tissues: III. A Comparison of Normal Hearts with Hearts Showing Congestive Heart Failure, J. Clin. Investigation **12**:1063, 1933. Calhoun, Cullen, Clark and Harrison.[8]

values. Seven patients may be classified as having acute infections (patients 82, 3, 8, 9, 20, 24 and 10). Five of these patients died of acute peritonitis and the remaining 2 of septicemia. The creatine, phosphorus and potassium in the left ventricle averaged 224, 201 and 291 mg., and in the right ventricle 181, 172 and 248 mg. respectively. No data are available on voluntary muscle. In general, these figures are somewhat above the average of the group of normal persons.

Patient 18, who showed definitely elevated values, should perhaps be classified with the group of patients with nitrogen retention, although there were no chemical data on the blood to substantiate this assumption.

In 3 patients with diabetes mellitus (patients 81, 65 and 62) the creatine, phosphorus and potassium averaged 200, 197 and 272 mg. in the left ventricle; 126, 144 and 194 mg. in the right ventricle, and 378, 183 and 286 mg. in voluntary muscle respectively. This was a definite decrease in the values for voluntary muscle and for the right ventricle, with practically normal values for the left ventricle. Another patient with diabetes mellitus (patient 83), whose case is not tabulated here, was included among the patients with uremia, because of nitrogen retention.

No explanation can be given for the elevated creatine values found in case 21.

COMMENT

The changes in the concentration of creatine, phosphorus and potassium in cardiac muscle associated with cardiac decompensation appear to be the most significant results of this study. It is evident that the relative losses of creatine, phosphorus and potassium are of such a magnitude as one might expect if these changes resulted chiefly from a breakdown of phosphocreatine. The argument that a decrease in the relative amount of active muscle, as a result of edema, infiltration of fat and fibrosis, is the cause of the decreased concentrations of creatine, phosphorus and potassium does not fit the experimental data. Analyses in which fat and water were also determined have shown that values calculated on a fresh weight basis agreed better with other findings than those calculated on a dry weight basis, unless the fat content was also included. The fat content of the heart was found to vary greatly, but was usually higher in the right ventricle than in the left ventricle.

The analyses reported here have confirmed the decrease in phosphorus and potassium observed by the investigators at Vanderbilt Medical School.[14] The decrease in total phosphorus has been supplemented by a smaller series of determinations of acid-soluble phosphorus. No significant variations were found to occur in the acid-insoluble fraction, but the acid-soluble phosphorus varied to a greater extent, and with 1 exception, in a patient with diabetes mellitus, fol-

lowed closely the creatine concentration. It would therefore appear that the variations encountered in phosphorus were the result of changes in the concentration of acid-soluble phosphorus. Since the completion of this study Decherd and Blum [15] have reported analyses of the left ventricle of the human heart in 36 cases in which there was heart failure and in 33 cases in which there was no heart failure. They found a definite decrease in the acid-soluble phosphorus but no change in the acid-insoluble phosphorus.

Burns and Cruickshank [3a] recently investigated the effects of asphyxia and fatigue on the mammalian heart. They found that in asphyxia there was a loss of phosphocreatine and of adenylpyrophosphate, which in complete asphyxia was approximately 80 and 60 per cent respectively. With fatigue in the presence of oxygen they found the situation reversed, with an approximate loss of 25 per cent of the phosphocreatine and 50 per cent of the adenylpyrophosphate. These findings are in substantial agreement with the results of our study. Since tissue anoxia and fatigue are the two conditions most probably associated with cardiac decompensation, these findings fit satisfactorily into the hypothesis that the loss of phosphorus and potassium results from the decomposition of the dipotassium salt of phosphocreatine and other organic phosphates.

A study of the phosphorus compounds of the dog's heart in aortic insufficiency has recently been completed by Mangun and Roberts.[16] In the late stages of insufficiency in 2 dogs a marked loss of adenylpyrophosphate, as well as phosphocreatine, was observed, amounting to 40 per cent of the total pyrophosphate and 20 per cent of the phosphocreatine normally present. Essentially no change occurred in the inorganic phosphorus and the ester phosphorus.

The most recent studies [17] in our laboratories on the human heart have included the estimation of the total extractive purines. Assuming that the purine determined is present in the living tissue largely in the form of adenylpyrophosphate, these studies indicate that adenylpyrophosphate is present in about half the molecular concentration of the phosphocreatine. Determinations of creatine, extractive purine nitrogen and total acid-soluble phosphorus have been made on 24 human hearts. In 6 instances of myocardial failure the creatine was decreased about 33 per cent, extractive purines about 15 per cent and phosphorus about 14 per cent below the average of the series. Creatine, extractive oxy-

15. Decherd, C. M., and Blum, J. E.: Phosphorus Fractions in Human Heart Muscle, Proc. Soc. Exper. Biol. & Med. **38**:341, 1938.

16. Mangun, G. H., and Roberts, J. T.: The Relationship of Phosphorus to Creatine in Cardiac Muscle, read at the Meeting of the Division of Biological Chemistry of the American Chemical Society, Boston, September 1939.

17. Mangun, G. H., and Myers, V. C.: Purine Content of Human Cardiac and Voluntary Muscle, J. Biol. Chem. **133**:lxii, 1940.

purine nitrogen and total acid-soluble phosphorus averaged 205, 35.5 and 106 mg. per hundred grams respectively in the left ventricle of the 24 hearts and 161, 30.0 and 91 mg. in the 6 hearts of patients with myocardial failure. While the change in purine content is not as great as that in creatine, still it does indicate that there may be a considerable loss of adenylpyrophosphate from the heart.

Whether the chemical changes observed in myocardial failure, viz., the fall in creatine, phosphorus, potassium and purines (adenylpyrophosphate) are contributory to or merely associated with this condition obviously cannot be determined on the basis of the data available. However, if the present theories regarding the energetics of muscle are correct, the fall in the content of these constituents must reduce the capacity for work.

SUMMARY

Creatine, phosphorus and potassium determinations have been carried out on the samples of muscle from the left and right ventricles of the hearts obtained at autopsy from human beings with pathologic conditions. In 48 instances analyses were also carried out on voluntary muscle.

The content of creatine, phosphorus and potassium in the left and right ventricles and in voluntary muscle averaged about 5 per cent less than in samples from 13 normal persons and varied considerably more.

In 17 patients with congestive heart failure, the creatine, phosphorus and potassium concentrations of both ventricles and of voluntary muscle were consistently decreased. The percentage decrease was greatest for creatine and least for phosphorus, while potassium occupied an intermediate position.

The average figures obtained for persons with heart failure showed a loss of 4.0 mols of creatine, 6.0 mols of phosphorus and 9.4 mols of potassium per kilogram. It is suggested that this loss is due primarily to a breakdown of dipotassium phosphocreatine with a subsequent loss of its components.

In uremia the creatine, phosphorus and potassium contents of voluntary muscle were found to be somewhat higher than the average values, 50 per cent of the values being higher than the normal range. Values for cardiac muscle were also relatively high. The increase in the creatine content of voluntary and cardiac muscle in uremia finds probable explanation in the retention of creatinine and the equilibrium which exists between creatinine and creatine. The changes in the phosphorus and potassium are probably related to the creatine changes.

The creatine, phosphorus and potassium values tended to be decreased in voluntary muscle in all conditions not associated with nitrogen retention, with the possible exception of fever and emaciation. Values in cardiac muscle, on the contrary, were usually normal or slightly elevated, unless heart disease was evident.

HEMOCHROMATOSIS

REPORT OF A CASE

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The following case is reported primarily because of (*a*) the absence of glycosuria and of significant decrease in dextrose tolerance in spite of the unusual quantity of iron in the liver and pancreas, (*b*) the absence of iron-containing pigment in the apparently characteristically pigmented skin, (*c*) the unusual complication of cutaneous xanthomatosis with hypercholesteremia in the absence of diabetes, (*d*) the antemortem demonstration of deficiency in adrenal cortical and androgenic hormones and (*e*) the unusual cause of death.

REPORT OF CASE

History.—S. S., a white Polish coal miner aged 45, was admitted to the service of Dr. H. A. Reimann, Jefferson Medical College Hospital, on Sept. 9, 1938, with the following complaints: (*1*) blue-gray discoloration of the skin (four years' duration), (*2*) intense itching (four years), (*3*) abdominal enlargement (three years) and (*4*) swelling of the legs (nine months).

The family history and past medical history had no bearing on the present condition.

Present Illness.—In July 1934 the patient first noted slight grayish blue pigmentation on the face and arms, which spread to the trunk and legs, increasing in intensity during the next two months. It varied little in intensity from August to the time of admission. Generalized itching began at the same time and persisted. Studies at another hospital on June 6, 1934 revealed the following conditions: deep slate color of the skin, with jaundice and enlargement of the liver and the spleen; icterus index, 100; Wassermann reaction, negative; basal metabolic rate, normal, and gastrointestinal roentgenogram, normal. The diagnosis on discharge was Banti's disease. Reexamination at the same hospital on Dec. 2, 1937 revealed, in addition to the aforementioned changes, rarefaction of the long bones, and it was noted on bronchoscopic examination that the mucous membrane of the pharynx, larynx and trachea was of essentially the same color as the skin. The diagnosis of biliary cirrhosis was made at that time. Abdominal enlargement was first noted by the patient in June 1935 and increased steadily. The appetite was poor, and there was constipation. There was a gradual loss of strength and energy. No sexual desire had been felt for the previous six years.

Physical Examination.—The skin over the entire body was deep slate blue with a bronze tint. The blue was most pronounced on the face and arms, and the pigmentation was most intense on the external genitalia. There was no abnormal pigmentation of the conjunctivas or of the lingual or buccal mucous membranes.

From the Department of Medicine and the Laboratories of Biochemistry and Pathology, Jefferson Medical College Hospital.

The lips were deeply pigmented (blue bronze), as was the mucous membrane of the lower part of the rectum. The scleras were yellow.

There were many xanthomatous lesions distributed over both upper eyelids, the flexor and extensor surfaces of the elbows, the palm of the right hand, the anterior and posterior axillary folds and the back of the chest. These lesions were slightly raised and dull lemon yellow and varied in longest diameter from about 2 to 10 mm. There were scratch marks over the entire surface of the body.

The hair was of fine texture and not abundant but had normal distribution. There was a complete but reducible left inguinal hernia. Both testes were small and of flabby consistency, the left being almost impalpable. The prostate was small.

The heart appeared normal. The lungs showed signs suggesting anthracosis of moderate degree. The abdomen was markedly distended, particularly on the left side. The liver extended 5 cm. below the costal margin in the right midclavicular line. The edge was hard and sharp and the surface was smooth and not tender. The spleen was smooth and hard and extended 14 cm. below the costal margin. There was no evidence of ascites. The fingers showed moderate clubbing, and there was bilateral Dupuytren's contracture. The temperature was 98 F.; the pulse rate, 85; the respiratory rate, 20, and the blood pressure, 100 systolic and 60 diastolic. There was no edema of the extremities, and the peripheral vessels were not unduly thickened. The eyegrounds were normal, showing no abnormal pigmentation.

Roentgen Ray Examination.—Roentgenograms showed a moderate degree of anthracosis of the lungs, diffuse demineralization of the entire skeleton, enlargement of the liver and spleen, widening of the duodenal loop and deformity of the duodenal cap.

Electrocardiographic Examination.—An electrocardiogram taken on Sept. 15, 1938 was normal.

Histologic Examination (Skin).—On September 23 microscopic studies showed an excess of melanin pigment in the normal situation. There was no evidence of iron-containing pigment. A section through a xanthomatous area showed characteristic cells of that type of lesion.

Laboratory Studies.—Many of the significant results of laboratory tests are presented in tables 1 and 2. Additional findings were as follows:

Urine (several examinations): One plus reaction for albumin and 2 plus reaction for bilirubin were obtained, but there was no dextrose, melanin, hemosiderin-containing cells or porphyrins. Volume 1,000 to 2,000 cc. daily. Fluid intake 1,500 to 2,500 cc. daily.

Bile and Feces: Bile obtained by duodenal drainage showed no significant abnormality. The feces contained a slight excess of neutral fat and fatty acids but were otherwise normal.

Gastric Analysis (Histamine): Free hydrochloric acid rose to a maximum of 6 and total acid to a maximum of 20 in forty-five minutes.

A sternal puncture on September 15 (Dr. Tocantins) showed no abnormalities.

A splenic puncture on September 15 (Dr. Tocantins) showed no abnormalities. No xanthoma cells were seen.

The basal metabolic rate on September 15 was + 16 per cent.

Course.—The patient left the hospital on November 4 and was readmitted, with the same complaints and with increased weakness, on Jan. 13, 1939. The results of physical examination were essentially the same as previously except that the liver (6 cm. below the costal margin) and the spleen (16 cm. below the costal margin) had apparently increased in size. The bronze tint of the skin was distinctly more pronounced, and there was slight pretibial edema.

Peritoneoscopic examination was performed on January 17 (Dr. K. Fry). The liver was enlarged and appeared to be of a gray-bronze color, with a finely granular surface. A piece was removed for study. The spleen was large and was covered by omentum, which prevented its direct visualization. Examination of the sections of the liver revealed an extensive multilobular type of cirrhosis, with large amounts of brownish, iron-containing pigment (hemosiderin) in a distribution characteristic of hemochromatosis.

The results of laboratory tests, in addition to those presented in tables 1 and 2, were as follows: The basal metabolic rate on January 28 was + 2 per cent and the urine was the same as on the previous admission. With the patient on a low calcium diet (110 mg. daily) and a moderate phosphorus intake (700 mg.), the three day urinary calcium excretion was 251 mg. and the phosphorus excretion 1,214 mg.

TABLE 1.—*Laboratory Data*

	9/12-3	9/24	10/12	10/25	1/16-17	2/21	2/28
Hemoglobin (per cent)	75	..	67	..	65	78	112
Red blood cells (per cu. mm.)	3,500,000	..	3,300,000	..	3,600,000	3,700,000	5,330,000
White blood cells (per cu. mm.)	5,400	..	2,650	..	2,120	3,300	13,800
Blood urea nitrogen	10.16	..	..	..	..	..	12.4
Urea clearance (per cent)	75	..	..	..	..	..	..
Serum calcium	9.36	..	..	..	10.2	..	..
Serum phosphorus	3.3	..	..	..	3.5	..	..
Serum phosphatase (units)	13.1	..	..	19.1	23.3	..	..
Serum protein (Gm.)	7.6	..	..	..	7.08	6.96	9.2
Serum albumin (Gm.)	4.16	..	..	..	3.07	3.28	..
Serum globulin (Gm.)	3.46	..	..	..	4.01	3.68	..
Serum chloride	..	..	..	..	375	..	362
Total cholesterol (mg.)	490	398	..	275	325	336	..
Ester cholesterol (mg.)	333	242	..	242	114	230	..
Free cholesterol (mg.)	157	156	..	33	211	106	..
Direct van den Bergh test	+	+	+	+	+	+	..
Serum bilirubin	5.6	1.9	5.8	5.1	7.0	3.2	..
Bromsulphalein retention (%)	10	0	40	50	80	20	..
Hippuric acid excretion (Gm.)	..	1.04	..	..	..	0.72	..
Urobilinogen in urine (dilution)	1:100	1:200	1:200	1:400	..	1:400	..

TABLE 2.—*Dextrose Tolerance*

Hours after 100 Gm. dextrose	Dextrose: Mg. per 100 Cc. of Blood							
	0	½	1	2	3	4	5	6
9/13/38	83	166	176	114				
1/23/39	70	114	157	164				
2/22/39	79	...	143	114	106	74	79	80

On the third morning of the Cutler test regimen (adrenal cortical function), the chloride concentration in the urine was 362 mg. per hundred cubic centimeters.

Sex hormone studies (Dr. A. Rakoff) on February 11 showed: gonadotropic hormone (blood and urine), normal or slightly deficient; estrogenic hormone (blood and urine), normal, and androgen (urine), deficient.

The patient complained of anorexia and profound weakness, both increasing progressively. Beginning on February 3 he was daily given the following medication: ascorbic acid, 100 mg.; nicotinic acid, 300 mg.; thiamine hydrochloride, 1 mg. (intramuscularly), and brewers' yeast (powdered), 90 Gm. His appetite and strength improved steadily during the next three weeks. The xanthomatous lesions became distinctly smaller, many of the smaller areas disappearing. The cutaneous pigmentation appeared to be distinctly less marked. The scars from the removal of skin for biopsy were not unduly pigmented.

On February 27 the patient complained of sudden, severe periumbilical pain. The abdomen was distended, but there was no rigidity and peristalsis was audible.

The next day at 11 a. m., he suddenly collapsed into a shocklike state, with extreme dyspnea, cyanosis and pulmonary edema. The blood pressure was 78 systolic and 56 diastolic, and the venous pressure was 50 mm. of water. There was exquisite tenderness over the liver and umbilical region. The patient was placed in an oxygen tent and received dextrose, saline solution and an extract of adrenal cortex intravenously, with no beneficial effect. Death occurred the same day, about twenty-four hours after the sudden onset of abdominal pain. The autopsy was performed by the late Dr. B. L. Crawford.

Gross Observations at Autopsy.—The skin of the entire body was dark steel gray, that over the face and neck being darkest. There were numerous small circumscribed yellow areas on the cutaneous surface, more numerous over the face and upper extremities than elsewhere. There was no edema. There was a large scrotal hernia on the left side and a smaller one on the right. There was moderate clubbing of the fingers.

Serous Cavities: There was a small amount of yellow, turbid fluid in the peritoneal cavity, with a small amount of fibrinous exudate on the visceral peritoneum. The omentum was adherent to the liver, the abdominal wall and the large hernial sacs on both sides of the scrotum. The pericardial cavity was normal. The left pleural cavity was practically obliterated by dense adhesions, and the right contained about 500 cc. of slightly turbid fluid.

Heart: The organ weighed 390 Gm. and showed no significant change.

Lungs: The left lung weighed 1,010 Gm.; the right, 850 Gm. Both were markedly anthracotic. On section, considerable blood and frothy fluid exuded from the cut surface. The tracheobronchial lymph nodes were enlarged, softened and anthracotic.

Spleen: The organ weighed 1,050 Gm. and was 26 by 14 by 6 cm. The capsule was markedly thickened, about two thirds of the anterior surface being hyalinized. The splenic pulp was rather firm and red and contained numerous small, darker red areas.

Adrenal Glands: The glands were rather soft but showed no other gross abnormality.

Kidneys: The left kidney weighed 150 Gm.; the right, 180 Gm. The capsule stripped readily, leaving a smooth surface. On section, there were numerous small yellow flecks in the cortex and medulla, most numerous in the pyramids. The relation between the cortex and the medulla was not altered.

Prostate: The gland was slightly enlarged and contained a small nodule in each lateral lobe.

Testes: The testes were small and compressed by the hernial sacs. On section, the gland structure appeared to be atrophied.

Gastrointestinal Tract: There was pigmentation of the duodenum and the first portion of the jejunum.

Liver: The organ weighed 3,170 Gm. and was 36 by 23 by 9 cm. It was irregular in outline and was adherent to the surrounding structures. It was firm, and the surface was finely nodular. On section, it appeared dark gray (slate colored), peppered with small yellow areas. The normal lobular markings were not apparent. There was no evidence of obstruction of the biliary passages (extrahepatic), but the gallbladder was greatly distended and filled with thin, light bile.

Pancreas: The pancreas weighed 250 Gm. Its consistency varied in different areas. In firmer portions the tissue was dark brown. Near the head there was an extensive area of necrosis and hemorrhage.

Abdominal Lymph Nodes: These were enlarged and dark brown.

Bone Marrow: The bone marrow of the right femur was soft, friable and putty-like.

Brain (Dr. B. J. Alpers): The weight was 1,230 Gm. The meninges were clear and translucent. There was a slight brownish discoloration over the cerebral hemispheres, and the cerebral vessels exhibited slight atherosclerosis. On section through the splenium of the corpus callosum a small bluish gray area was noted in the centrum ovale of the right cerebral hemisphere. There was also some grayish discoloration dorsal to the right pyramid on section through the pyramidal decussation. The hypophysis was grossly normal.

Other organs: The thymus, thyroid, larynx, bladder and ureters appeared normal.

Microscopic Observations at Autopsy.—The following were the significant histologic observations:

Skin: There were an increase in melanin in the normal situation, some fibrosis of the true skin and focal areas of xanthomatous change. There was no evidence of hemofuscin or of iron-containing pigment (berlin blue stain).

Thyroid: There was a small amount of brownish pigment, which reacted positively with the iron stain.

Lung: The pleura was thickened and infiltrated with carbon. The alveolar walls were somewhat thickened, and anthracotic pigment was deposited throughout the lungs.

Heart: There was no significant change. Careful study revealed no significant deposition of pigment about the nuclei of the muscle fibers.

Spleen: The capsule was markedly thickened. The malpighian corpuscles were somewhat atrophic, and the centers were filled with blood. There was proliferation of the fibrous tissue, and the sinusoids stood out prominently, owing to their engorgement with blood. There was an extensive deposition of iron-reacting pigment throughout the organ, principally in the cells lining the sinusoids.

Liver: The capsule was greatly thickened. There was a marked increase in the perilobular connective tissue, with some invasion of the lobules. The latter were much diminished in size, and there was widespread degeneration of the hepatic cells. There was an attempt in some areas at regeneration of bile ducts and liver cells. There were small foci of inflammatory reaction throughout the fibrous tissue, which contained an enormous amount of brown, iron-reacting pigment, which was also present in the hepatic cells and, to a larger extent, in the Kupffer cells.

Pancreas: There was considerable postmortem change. However, no areas of fat necrosis were observed. There was a slight increase in fat and in interlobular fibrous tissue. The most marked feature histologically, apart from the area of necrosis and the hemorrhage noted grossly, was the extensive infiltration of the pancreatic tissue with a brown, iron-reacting pigment.

Adrenal Glands: There was extensive deposition of iron-reacting, brown pigment in the cortex.

Pituitary Gland (Dr. B. J. Alpers): Large amounts of granular, iron-containing pigment were seen in the cells of the anterior lobe.

Testes: Slight atrophy of the tubules was noted. A moderate amount of brown, iron-reacting pigment was present in the interstitial tissue.

Retroperitoneal Lymph Nodes: The sinuses were dilated. There was an enormous amount of iron-reacting, brown pigment, which obscured much of the histologic picture.

Brain (Dr. B. J. Alpers): Large amounts of granular, iron-reacting pigment were present in the epithelium of the choroid plexus.

Chemical Studies of Tissue.—Pieces of tissue were taken for chemical analysis, the iron being determined by the method of Farrar [1] and the final determination being made colorimetrically according to the method of Kennedy.[2] The iron content of the various tissues is presented in table 3.

TABLE 3.—*Iron Content of Tissues (Grams per Hundred Grams Dry Weight)*

Organ	Weight, Gm.	Iron, per Cent Dry Weight	Total Iron, Gm.
Liver	3,170	7.23	45.33
Pancreas	250	5.84	3.52
Retroperitoneal lymph node	.....	15.31	
Thyroid	.....	0.71	
Spleen	1,050	0.50	1.26
Heart	390	0.20	
Skin	.....	0.06	
Adrenal	.....	0.32	
Small intestine	.....	0.13	
Kidney	330	0.19	
Testis	.....	0.09	
Skeletal muscle	.....	0.05	
Liver copper, 0.004 Gm. per 100 Gm. dry weight			

COMMENT

This case presents several unusual features. When the patient was first seen, the diagnosis of hemochromatosis was suggested immediately by the apparently characteristic color of the skin and the presence of an enlarged, hard liver. However, certain other observations cast doubt on this diagnosis, which, in fact, was not established until the second admission to the hospital, about six weeks before death. These confusing facts were: (*1*) the absence of glycosuria and the only slightly abnormal dextrose tolerance curves, (*2*) the presence of cutaneous xanthomatosis and hypercholesteremia, (*3*) the absence of iron-containing pigment from the apparently typically pigmented skin and (*4*) the relatively marked degree of splenomegaly. These discordant features, with the hyperbilirubinemia, led to the tentative diagnosis of xanthomatous biliary cirrhosis (Thannhauser and Magendantz [3]). This condition is supposedly dependent on, or at least associated with, biliary obstruction due to xanthomatous lesions in the extrahepatic bile passages. Two features present in this case were not in complete accord with this diagnosis: (*1*) the definitely blue-bronze color of the skin, particularly of the face, and (*2*) the relatively slight degree of retention of bromsulphalein

1. Farrar, G. E.: The Determination of Iron in Biological Materials, J. Biol. Chem. **110**:685, 1935.

2. Kennedy, R. P.: The Quantitative Determination of Iron in Tissues, J. Biol. Chem. **74**:385, 1927.

3. Thannhauser, S. J., and Magendantz, H.: The Different Clinical Groups of Xanthomatous Diseases: A Clinical Physiological Study of Twenty-Two Cases, Ann. Int. Med. **11**:1662, 1938.

(10 per cent) on admission as compared with that of bilirubin (5.6 mg.). In our experience, this is unusual in obstructive jaundice. However, varying degrees of pigmentation of the skin were reported by Thannhauser and Magendantz [3] in xanthomatous biliary cirrhosis, and it is conceivable that the hyperbilirubinemia might be dependent largely on hepatocellular damage rather than on biliary obstruction. Under these circumstances this discrepancy between dye and bilirubin retention is not uncommon. No xanthoma cells or other lipid-containing cells could be found in material obtained by sternal and splenic puncture.

The diagnosis of hemochromatosis was established by histologic examination of a piece of liver removed at peritoneoscopic examination. This necessitated reinterpretation of the observations made previously and led to further studies that might be of interest in this condition. The subsequent course and the observations at necropsy, although confirming the diagnosis, did not explain certain of the puzzling and unusual manifestations presented by this patient. These, with certain additional interesting observations, will be discussed individually.

Absence of Glycosuria.—The absence of glycosuria is not in itself a rare phenomenon in cases of hemochromatosis. According to Sheldon,[4] glycosuria was absent in 22 per cent of 236 cases collected from the literature. In the majority of these, however, no studies were made of the dextrose tolerance, and it is probable that a state of latent diabetes was present in many instances. This was true in at least 3 cases (Evans,[5] Sheldon [6] and Yater, McNabb and Horgan [7]). Three dextrose tolerance tests were performed in the present case. The fasting blood sugar concentrations were normal. The first curve (Sept. 13, 1938) suggested slightly diminished tolerance; the second (Jan. 23, 1939), more marked impairment, and the third (February 22), very slight impairment. The first and third curves were of the type not infrequently observed in patients with hepatocellular damage of the extent present here and could be explained on this basis alone.

The extensive acute degeneration of the pancreas incident to the terminal episode unfortunately precludes any accurate estimate of the extent of previous change in the islands of Langerhans. However, according to Sheldon,[8] diabetes appears to be present invariably in cases in which the concentration of iron in the pancreas is above 1.5 per cent, increasing in severity as the quantity of pigment increases. On this

4. Sheldon, J. H.: Haemochromatosis, London, Oxford University Press, 1935.

5. Evans, G.: Bronzed Diabetes, Proc. Roy. Soc. Med. **24**:477, 1931.

6. Sheldon, J. H.: Haemochromatosis, Lancet **2**:1031, 1934.

7. Yater, W. M.; McNabb, P. E., and Horgan, E.: Clinico-Pathologic Types of Haemochromatosis, M. Ann. District of Columbia **1**:28, 1932.

8. Sheldon (footnotes 4 and 6).

basis, diabetes should certainly be expected with an iron content in the pancreas of 5.84 per cent. In the absence of information regarding the condition of the islet cells, no satisfactory explanation for the absence of glycosuria and of significantly decreased dextrose tolerance (except temporarily) can be offered. However, in the light of the evidence of adrenal cortical insufficiency, as indicated by the supernormal chloride concentration in the urine in the test proposed by Cutler,[9] the possible influence of this factor in mitigating or preventing diabetic manifestations should receive consideration. The usually heavy pigmentation of the anterior lobe of the hypophysis suggested to Sheldon [4] that defective function of this organ may be implicated in the mechanism of increased sensitivity to insulin frequently exhibited by patients with hemochromatosis. In view of present knowledge of the role of the adrenal cortex in carbohydrate metabolism, the observation recorded here may have a similar significance.

Hypogonadism and Hypocorticoadrenalism.—Hypogonadism, with loss of sexual desire, impotence and loss of hair, with alteration in its texture (soft, fine and silky), occurs rather frequently in hemochromatosis. According to Sheldon,[4] it probably ranks next to the classic triad of symptoms in order of frequency. As in this case, the testes are usually small and soft, and atrophy of the germinal epithelium is frequently observed. Labbé and his associates [10] applied the designation "hepatopancreaticogenital syndrome" to this phenomenon occurring in association with hemochromatosis. They attributed the changes in secondary sex characteristics to those in the testes, the cause of the latter being unknown. It is interesting that these organs are usually not heavily pigmented.

As pointed out by Sheldon,[4] the rather constant occurrence of pigmentation of the anterior lobe of the hypophysis and the adrenal cortex suggests that hypofunction of one or both of these glands may be of fundamental importance in this connection. This hypothesis was supported by his observation in two instances that the basophils of the anterior lobe of the hypophysis were much more deeply pigmented than the eosinophils. In the present case, there was a large amount of hemosiderin in this situation. However, the observation of a distinct deficiency in excretion of androgen, in conjunction with an essentially normal or, at most, only slightly deficient quantity of gonadotropic hor-

9. Cutler, H. H.; Power, M. H., and Wilder, R. M.: Concentrations of Sodium Chloride and Potassium in the Urine and Blood: Their Diagnostic Significance in Adrenal Insufficiency, J. A. M. A. **111**:117 (July 9) 1938.

10. (*a*) Labbé, M.; Boulin, R.; Azerad, E., and Uhry: Diabète bronzé avec infantilisme tardif, Bull. et mém. Soc. méd. d. hôp. de Paris **47**:542, 1931. (*b*) Labbé, M.; Boulin, R., and Petresco, M.: Etude histologique d'un cas de cirrhose pigmentaire du foie avec diabète et infantilisme régressif, ibid. **48**:440, 1932.

mone in the blood and urine, suggests that the hypogonadism is perhaps not of hypophysial origin. No definite statement can be made, on the basis of the findings in this case, of the underlying cause of the hypogonadism, but the demonstration of evidence of corticoadrenal hypofunction raises the question as to whether this factor may be related to the testicular changes.

Several authors have attributed certain of the manifestations of hemochromatosis to an associated state of adrenal cortical insufficiency, emphasizing the resemblance to features of Addison's disease. Chief among these are (*1*) the brownish discoloration of the skin, with an increase in melanin in the malpighian layer, (*2*) weakness and (*3*) hypotension. The last is perhaps in part contributed to by the accumulation of pigment in the cardiac muscle fibers. In the present case, the average blood pressure was about 100 systolic and 60 diastolic, with no clinical, electrocardiographic or histologic evidence of significant myocardial damage. The iron content of the heart muscle (0.2 per cent) was distinctly increased (average normal 0.04 per cent) but was not very high as compared with values reported for this condition. The observation of a urinary chloride concentration of 362 mg. per hundred cubic centimeters, under conditions of the test procedure described by Cutler,[9] suggests the presence of a state of impaired function of the adrenal cortex. At the time of the terminal collapse, with marked fall in blood pressure, low venous pressure and evidence of hemoconcentration, the possibility of a crisis of adrenal insufficiency was considered, but active therapy with dextrose, sodium chloride and extract of adrenal cortex was without effect. The normal urea nitrogen content of the blood and the chloride content of the plasma at that time were not in accord with this diagnosis. The demonstration of evidence of cortical functional impairment seems significant in view of the heavy deposition of pigment in this situation.

Hypercholesteremia and Xanthomatosis.—These conditions have been rarely observed in association with hemochromatosis, and, as stated by Sheldon,[4] when hypercholesteremia does occur it is probably attributable to diabetes. Abel, Girard and Kissel [11] and Dvorak [12] reported extremely high values, the former 1,180 mg. and the latter 1,025 mg. per hundred cubic centimeters. It is interesting to note that the patient reported by Dvorak [12] presented some features identical with those in the present instance, viz., icterus, hypercholesteremia, xanthomatosis and pigmentation of the skin, the iron content of which was apparently

11. Abel, E.; Girard, J., and Kissel, P.: Étude anatomo-clinique d'un cas de diabète bronzé, Rev. méd. de l'est **61**:1, 1933.

12. Dvorak, R.: Zur Kenntnis der Hämochromatose, München. med. Wchnschr. **76**:743, 1929.

not increased. This case is not accepted as a genuine one of hemochromatosis by Sheldon,[4] nor could ours have been in the absence of opportunity to study tissues other than the skin. Becker [13] reported a case of hemochromatosis associated with so-called Schamberg's disease (progressive pigmentary dermatosis with a high cholesterol content of the blood), in which the cholesterol content of the plasma was 296 mg. per hundred cubic centimeters. However, the characteristic features of that condition were not present in the case described here (diffuse, yellow-brown discoloration, erythematous puncta and thinning of the epidermis, with disappearance of the interpapillary processes).

These manifestations (hypercholesteremia and xanthomatosis), occurring in the absence of glycosuria, significantly decreased dextrose tolerance and demonstrable deposits of hemosiderin or hemofuscin in the skin or epithelial cells in the urinary sediment and in the presence of icterus and splenohepatomegaly, led to a presumptive diagnosis of xanthomatous biliary cirrhosis. This was, of course, abandoned later. The cause of this unusual complication and its possible relation to the hemochromatosis remain in doubt. There was no obstruction of the extrahepatic bile passages, and in our experience xanthomatosis and hypercholesteremia with a normal total cholesterol–cholesterol ester ratio are rarely observed in portal cirrhosis or in hepatocellular types of jaundice. It may be pointed out that this ratio, previously normal, fell to 35 per cent (January 16) simultaneously with other evidence of increased impairment of hepatic function (table 1).

Cutaneous Pigmentation.—Neither absence of pigmentation nor absence of iron-containing pigment in the skin is rare in hemochromatosis (15 to 20 per cent). This subject has been discussed in detail by Sheldon,[4] who stated the belief that whereas the bronze color may be due merely to an increase of melanin in the malpighian layer, as in the present case, the metallic blue type of pigmentation is more likely to be associated with the deposition of hemosiderin in superficial layers of the skin. The latter could not be demonstrated in this patient, despite the distinctly blue color. This, however, was most pronounced in the skin of the face, the tissue of which was not examined histologically. The iron content of the skin was, however, increased. It is noteworthy also that, contrary to the usual finding, scars on the skin were only slightly pigmented.

Decrease in the intensity of pigmentation is not uncommon, either spontaneous or following administration of insulin. Consequently, no special significance can be attributed to the progressive diminution in pigmentation observed shortly after the administration of ascorbic acid

13. Becker, S. W.: Schamberg's Disease Associated with Hemochromatosis, Arch. Dermat. & Syph. **24**:380 (Sept.) 1931.

and vitamin B complex was begun. It is interesting, however, that appetite and strength improved simultaneously and that the xanthomatous lesions decreased in size, some disappearing completely. No attempt was made to eliminate cholesterol from the diet, which was high in protein and carbohydrate and low in fat. There have been several references recently to decrease in the intensity of pigmentation in Addison's disease following the administration of ascorbic acid,[14] and the relation of the latter to the metabolism of melanin and its precursors has been demonstrated.[15] The influence of ascorbic acid and the vitamin B complex on xanthomatous lesions merits further investigation.

General Features.—The spleen was unusually large (1,050 Gm.). In the series of 311 cases collected by Sheldon [4] the spleen ranged in weight from 114 to 950 Gm., the average being 416 Gm. The iron content of the liver and pancreas was also unusually high, the former containing about 45 Gm. (7.23 per cent dry weight) and the latter about 3.5 Gm. (5.84 per cent dry weight). The average iron content of the liver in 42 cases reviewed by Sheldon [4] was 21.36 Gm. (highest 38.7 Gm.). Incidentally, the copper content was not significantly high (0.004 per cent). As is true in the majority of such cases, the highest concentration of iron was found in the retroperitoneal lymph nodes (15.3 per cent). One unusual finding, in view of the enormous amounts of iron present elsewhere, was the relatively low iron content of the skeletal muscle (psoas) and the heart muscle, about two and five times normal, respectively (table 2).

The cause of death was apparently unrelated to the condition of hemochromatosis, in which death is usually due to diabetic coma (50 per cent), cirrhosis (11 per cent), pneumonia (10 per cent), tuberculosis (9 per cent), carcinoma of the liver (7 per cent), myocardial failure and intercurrent infection. Autopsy revealed extensive necrosis of the pancreas. There was no evidence of fat necrosis, and no cause for the lesion could be demonstrated. Clinically, the acute terminal episode suggested a crisis of adrenal insufficiency or some abdominal catastrophy, and, viewed in retrospect, acute pancreatic necrosis should have been suspected.

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14. Cantarow, A.: Vitamins, Internat. Clin. **1**:221, 1940. Cornbleet, T.: Vitamin C and Pigment, Arch. Dermat. & Syph. **35**:471 (March) 1937. Morawitz, P.: Pathologische Hautpigmentierung und "Pigmentvitamine," Klin. Wchnschr. **13**:324, 1934.

15. Sealock, R. R.; Ziegler, B., and Driver, R. L.: The Relation of Vitamin C to the Metabolism of the Melanin Pigment Precursors, Tyrosine and Dihydroxyphenylalanine, J. Biol. Chem. **128**:lxxxix, 1939. Sealock, R. R., and Silberstein, H. E.: The Control of Experimental Alcaptonuria by Means of Vitamin C, Science **90**:516, 1939. Levine, S. Z.; Marples, E., and Gordon, H. H.: A Defect in the Metabolism of Aromatic Amino Acids in Premature Infants: The Role of Vitamin C, ibid. **90**:620, 1939.

SUMMARY

A case is presented of hemochromatosis without glycosuria or iron-containing pigment in the skin, which, however, was blue-bronze in color. The absence of these features, together with the presence of cutaneous xanthomatosis and hypercholesteremia and hyperbilirubinemia, caused considerable difficulty in diagnosis. The true nature of the condition was established by examination of a piece of liver removed at peritoneoscopic examination. Evidence was obtained during life of deficiency in adrenal cortical hormone and in androgen. The possibility was suggested that the diabetic tendency which might otherwise have been present might have been mitigated by the existing state of hypocorticoadrenalism. Chemical examination of the tissues revealed enormous quantities of iron in the liver, pancreas and retroperitoneal lymph nodes and relatively little in the heart and skeletal muscle. Death was due to the unusual complication of acute pancreatic necrosis.

ESTIMATION OF RENAL FUNCTION WITH THE AID OF AN IODOSECRETORY INDEX AND A NEW UREOSECRETORY INDEX

AN IMPROVED APPLICATION OF THE CREATININE METHOD FOR DETERMINING DIURESIS

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It is the object of the examinations and observations described in this article to obtain more accurate results in the estimation of renal function.

For this purpose it is recommended to use an iodosecretory index, obtained after administering an iodide. Should this not be possible or not be allowed, it is advised to replace this iodosecretory index by a new ureosecretory index. Both indexes have been deduced from the improved laws of Ambard, and the manner in which this has been done is described.

In 1919 I described a formula for the application of Ambard's laws in the diagnosis of renal diseases. This formula had certain advantages over the formula deduced by Ambard himself from his laws.[1] It appeared, however, that the new formula was endowed with some hereditary taint, for in 1921 Austin, Stillman and Van Slyke,[2] examiners at the Rockefeller Institute of New York, established the fact that one of Ambard's laws is inaccurate. From this discovery it follows that all the formulas deduced from Ambard's laws must be considered worthless. This also applies to McLean's index, in which Ambard's coefficient was converted in such a manner that the value taken as a norm was 100. In the following pages I shall try to prove that also the urea clearance test deduced from the improved laws of Ambard and set up by the aforementioned examiners not seldom produces unreliable results and, further, that with the aid of these improved laws more reliable formulas can be set up when urea is replaced by iodine. The "iodosecretory index" recommended has produced more reliable results, and the chemical examination according to iodine determination described in this article is simpler and takes up less time than the urea determination.

1. Ambard, L., and Weill, A.: J. de physiol. et de path. gén. **14**:753, 1912.

2. Austin, J. H.; Stillman, E., and Van Slyke, D. D.: J. Biol. Chem. **46**:91, 1921.

The conviction is growing that for determination of renal function all one-sided methods of examination, i. e., all methods in which the blood alone or the urine alone is examined, depend too much on extrarenal influences. No such method has escaped this source of mistakes; neither Korányi's cryoscopic test, nor Strauss's rest nitrogen determination,[3] nor Widal's [4] blood urea determination, nor Volhard's concentration and dilution test,[5] nor Schlayer's potassium iodide or lactose test,[6] nor Rowntree's phenol red test [7] nor any of the many other tests with provoked eliminations. I saw that a great deal of time was lost in all these tests, of which it is now ascertained that their reliability does not justify the enthusiasm with which they were welcomed in many clinics. I have no intention to enter here into further details, but I refer the reader to the critical views of Chabanier and Lobo-Onell,[8] which are in accordance with my experience. These authors strongly recommended the combined methods of examination, i. e., the methods in which parts of blood and urine are taken in mutual correlation. The time has come to control always the one-sided methods by means of combined methods. Nobody will deny the greater advisability of spending more time on a reliable examination than of applying so-called simple methods which, in many cases, even if applied with all possible corrections, cannot stand scientific criticism. A thing worthy of note is that Chabanier and Lobo-Onell were by no means blind to the mistakes entailed by clinging to the combined method, called the constant, or coefficient, of Ambard, recommended by them. They acquainted themselves with the aforementioned improvements of Ambard's laws, and they moreover pointed out the frequent occurrence of a relative oliguria with regard to urea, which makes all ureosecretory constants worthless. Yet, unconceivably, they kept recommending Ambard's coefficient. Probably the reason is that the application of a combined method with the aid of a sulfate or an iodide (with which they were also acquainted) met with practical difficulties. These methods required chemical determinations, which took much time and which must be left to a professional chemist. To surmount these difficulties the firm of Hellige has made, on my request, special sliding glasses by means of which determinations of the level of iodine in blood and urine can be made in a manner sufficiently accurate for clinical purposes.[9]

3. Strauss, H.: Die chronischen Nierentzündungen in ihrer Einwirkung auf die Blutflüssigkeit, Berlin, A. Hirschwald, 1902.

4. Widal, F., and Javal, A.: Compt. rend. Soc. de biol. **57**:301, 1904.

5. Volhard, F.: Verhandl. d. deutsch. Kong. f. inn. Med. **27**:735, 1910.

6. Schlayer and Takayashi: Deutsches Arch. f. klin. Med. **101**:333, 1911.

7. Rowntree, L. G., and Geraghty, J. T.: Tr. Am. A. Genito-Urin. Surgeons **5**:59, 1910.

8. Chabanier, H., and Lobo-Onell, C.: Exploration fonctionelle des reins, Paris, Masson & Cie, 1930.

9. See note at end of article.

In a number of cases it is difficult (practically speaking, even impossible) to establish whether during the examination there will be a relative oliguria with regard to urea. For this reason it is advisable as a rule to determine the iodosecretory index. In several cases I was led astray by the ureosecretory index. This index may be reserved for cases in which administration of iodine preparations is not advisable or not allowed (e. g., cases of exophthalmic goiter), for cases in which a relative oliguria with regard to urea can be excluded with certainty or for those in which, for want of the necessary means to determine the level of iodine in blood and urine, one has to make the more cumbersome urea determinations. For instance, in a case of disease of the kidneys when there are disturbances of circulation as well, it is very difficult to establish whether a relative oliguria with regard to urea can be excluded. Clinically speaking, oliguria in general is sometimes out of the question, and yet there is a relative oliguria with regard to urea, because the maximum concentration for urea secretion has strongly diminished in consequence of the disease process in the renal parenchyma. Thus a diuresis of more than 1.5 liters can be attended with relative oliguria with regard to urea. This fact is too often overlooked. In 1919, Ambard and Papin [10] pointed out this fact. There no longer exists free play between urea in blood and urea in urine when the latter has reached its maximum concentration. With healthy kidneys this happens at a level of urinary urea of about 50 to 55 Gm. per liter, but with pathologic kidneys this value may fall to 10 Gm. per liter or less. Inaccurate results of the determinations with Van Slyke's urea clearance test were often obtained in cases of relative oliguria with regard to urea. This relative oliguria also deserves consideration in cases of edema, whether the edema is due to cardiac or to renal disease. Sometimes a relative oliguria with regard to urea may be based on a nervous condition, as I have been able to observe. All this makes one doubt whether in a certain case a relative oliguria with regard to urea does not render useless the trouble taken in making accurate determinations with Van Slyke's urea clearance test. It is an ascertained fact that a deficiency of salt in connection with hyperazotemia is not founded on some fantastic defense reaction, as Blum and his associates [11] contended, but is founded on a relative oliguria with regard to urea. The fact that every substance secreted by the kidneys has a certain maximum concentration and that the maximum concentrations of different substances are isomolecular made examiners look for substances the molecular weight of which is great enough to obviate the aforementioned difficulties caused by urea with its relatively low molecular weight. Moreover, it is known that the

10. Ambard, L., and Papin, E.: Arch. internat. de physiol. **8**:437, 1919.
11. Blum, L.; Grabar, P., and Van Caulaert, C.: Ann. de méd. **25**:34, 1934.

maximal concentrations for the different substances are dependent on the renal parenchyma but are independent of other substances secreted by the kidneys at the same time. To establish a limit value in a certain case (e. g., for urea by administering large quantities of urea or of milk) is not only troublesome for the patient but cannot be carried out in many cases on account of nausea or polyuria. Such methods must be left out of consideration.

Before entering into details concerning the iodosecretory index, I shall discuss a few objections raised against these methods of examination. Then I shall explain the improvements which I made in setting up the formulas. These improvements apply to both the iodosecretory and the ureosecretory index.

In 1918, Addis [12] subjected Ambard's laws to a severe criticism. He made extensive examinations in order to compare the secretion of blood urea and of urinary urea under different conditions. In 1921, Austin, Stillman and Van Slyke [2] ascertained the fact that one of Ambard's laws is inaccurate. In contrast with Addis' observations, they were of the opinion that the proportion between urea secretion and urine volume is altered when the diuresis has reached a certain limit. This limit they called the "augmentation limit." Addis and Drury [14] opposed this opinion, but the results of my own examinations of the same persons, made repeatedly at short intervals, were consistent only if I took into account the existence of an "augmentation limit." In the opinion of the physiologist Peters,[15] the significance of the "augmentation limit," is still not clear. Holten and Rehberg [16] raised a few objections against the use of urea clearances, but their criticism does not apply to the procedure described by me.

I shall not adopt the name "urea clearance" in the following discussion, because the name "ureosecretory index," used by me [17] in a previously published article, speaks for itself, whereas the expression "urea clearance" needs an explanation, as I know from experience.

Without entering into detail regarding the mistakes made in applying the ureosecretory index, I cannot refrain from pointing out the inaccuracy entailed by giving much fluid or much meat very shortly (e. g., within an hour) before the examination. By the tests of Weil it became a well known fact that when this is done the value for serum urea at the

12. Addis, T.: Am. J. Physiol. **46**:1, 1918.

13. Footnote deleted.

14. Addis, T., and Drury, D. R.: J. Biol. Chem. **55**:639, 1923.

15. Peters, J. P.: Body Water: The Exchange of Fluids in the Body, Springfield, Ill., Charles C. Thomas, Publisher, 1935, p. 263. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, Williams & Wilkins Company, 1932.

16. Holten, C., and Rehberg, P. B.: Acta med. Scandinav. **74**:479, 1931.

17. Peters, J. T.: Arch. f. klin. Med. **129**:3, 1919.

end of the test often shows a difference of 40 per cent from the value observed at the beginning of the test. This fluctuation in the level of blood urea necessarily results in great fluctuation in the concentration of urea in the urine during the test period. It is only accidental if the average concentration of urea in the urine secreted by the kidney during the test period is equal to that in the urine which passed the kidney at the very time of the blood drawing (with which, in point of fact, one should compare the value for serum urea in order to have the most reliable result for the ureosecretory index). During the test period the curve of secretion of urea in urine proceeds in an irregular manner, with transitory rises and falls. Therefore, drawing blood exactly halfway through the test period does not guarantee reliable results. If chance cooperates, the results are reliable; if, however, chance thwarts one, the results are worthless. Hence it

TABLE 1.—*Effect of Recent Ingestion of Meat, Fish, Eggs or Milk* *

Patient	Examination	Column A		Column B	
		Ur	U. I.	Ur	U. I.
Miss B.	1st	0.4	82	0.53	87
	2d	0.36	89	0.465	88
Mr. K.	...	0.6	64	0.58	63
Mrs. N.	1st	0.74	60	0.78	58.5
	2d	0.71	55	0.82	58
Miss W.	...	0.545	41	0.7	46
Mr. B.	...	0.6	58	0.67	58

* In column A are mentioned the values for blood urea in grams per liter (Ur) and the ureosecretory index (U. I.), when for twelve hours no meat, fish, eggs or milk had been taken. In column B the same values are mentioned, when two hours before the examination 200 Gm. of beefsteak had been taken. The interval between the first and the second examinations varied from a few days to a few weeks.

is necessary to make such preparations before starting the examination as will prevent great fluctuations of the concentration of urea in the urine during the test period. However, it is unnecessary, as it was formerly advised, to make the examination in the morning before breakfast, unless one wishes to know at the same time the value of serum urea while it is less influenced by previously ingested food. According to my experience, if one sees to it that during five hours before the test moderate quantities of fluid are taken and that neither food nor fluid is taken during the hour preceding the test, reliable results will be obtained. From tests (table 1, column B) which I began to make one hour after 200 Gm. of meat had been taken it is seen that the ureosecretory index, even after such an overloading with albumin, deviates strikingly little from values observed when, twelve hours previously, a diet poor in nitrogen had been observed (table 1, column A). All this holds true on condition that no relative oliguria with regard to urea exists. In all cases only the value for serum urea has risen above normal limits. This proves once more that determination of this value only is insufficient to test

renal function. It needs no further demonstration that, for the same reason, in determining the iodosecretory index stronger fluctuations of the concentration of iodine in blood and urine should be prevented after administration of an iodide.

Some have made the remark that they could not understand how the quantity of renal tissue, an anatomic value, can affect the function of the kidney. Of course, cases in which one part of the kidney has received a local injury and in which by consequence another part shows compensatory hyperfunction must be excluded. This I shall discuss later. However, in normal renal parenchyma, when comparison is made between the diuresis of adults and children, other things being equal, the normal diuresis of healthy kidneys is seen to be reduced in proportion to the decrease in weight of the renal parenchyma. This is acknowledged by most writers, such as Ambard, Addis and Conway. As determination of the weight of the renal parenchyma proved to be too difficult, they inserted into the formula the factor of body weight, supposing that the body weight ran parallel to the weight of the renal parenchyma. Now in this lies a mistake, made by Ambard and Conway. It is a still greater mistake to take no account of the renal weight, as in the simplified formula of Van Slyke's urea clearance. In his unsimplified formula Van Slyke introduced the factor "surface area," which was supposed to be proportional to the amount of kidney tissue. However, one can be sure that in many hospitals the simplified urea clearance is used, since I find in the latest editions of widely read and excellent books (Todd and Sanford;[18] Koch;[19] Levinson and McFate[20]) only the simplified formula. (Osgood[21] described the urea clearance with the factor "surface area.") With neglect of the amount of kidney tissue, a deviation of renal function can be wrongly diagnosed in the case of a small person or a child, because the subject has less renal parenchyma than an adult of normal size and consequently shows a slighter diuresis. With renal weights above the average, a deviation can be camouflaged. When the weight of the renal parenchyma is replaced by the body weight, the mistake is most evident with sufferers from adiposity or macies and also in a high degree with children. That it is not permissible to assume that body weight runs parallel to renal weight I can easily show with the aid of a curve, in which renal weight

18. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis, ed. 8, Philadelphia, W. B. Saunders Company, 1935.

19. Koch, F. C.: Practical Methods in Biochemistry, ed. 2, Baltimore, William Wood & Company, 1937.

20. Levinson, S. A., and McFate, R. P.: Clinical Laboratory Diagnosis, Philadelphia, Lea & Febiger, 1937.

21. Osgood, E. E.: Laboratory Diagnosis, ed. 3, Philadelphia, The Blakiston Company, 1940.

is indicated in percentage of body weight (figures in left column) at different ages (chart 1). If renal weight and body weight should increase to the same degree in the period of growth, the same proportionate number, expressed in percentage between renal weight and body weight, should be found at all ages, and the curve in chart 1 should show a horizontal line. The enormous deviation from the horizontal points out the error made by inserting body weight instead of renal weight into the formulas.

In the case of children I refer the reader to anatomic atlases for the striking relative largeness of the kidneys. From this it can be concluded at first sight that the ratio of renal weight to body weight and also to surface area is altered during the period of growth. Consequently the calculation of Van Slyke's urea clearance also with the

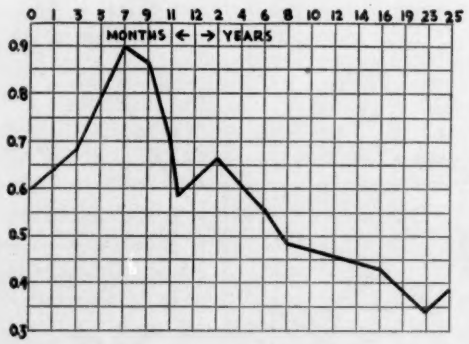

Chart 1.—Renal weight in relation to body weight. The horizontal column of numbers indicate the age of the subject; the vertical column of numbers indicates what percentage of body weight corresponds at a certain age with the weight of the kidneys.

factor "surface area" can produce unreliable results in the cases of children. Since anatomic observations, especially in children, refute the conception that the amount of kidney tissue is proportional to the surface area, I tried to solve the problem in another way.

The question is how the weight of the renal parenchyma can be calculated in the simplest manner. For this purpose I have noted the renal weight and the body height and weight at 100 postmortem examinations of adults and children. Instead of the renal weight, I have calculated the weight of the renal parenchyma by subtracting the average weight of the blood, the blood vessels and the basin of the kidneys. By this method the renal weight is reduced by one fifth on an average. It appears that with persons over 18 years of age the weight in grams of the renal parenchyma may be equated as 1.4 times the body height in

centimeters. I found it the simplest method to use the body height as a standard, for with sufferers from adiposity or macies this is still the most reliable standard. With persons under 18 years of age it is advisable to read the weight of the renal parenchyma from a chart made up by me for that purpose (chart 2) because after the age of 12 the quantity of renal parenchyma increases more in girls than in boys. In girls after the age of 14 it again lags behind as compared to that of boys. With this method a simple multiplication is sufficient for adults and the use of a simple curve for children. With this method one escapes also the

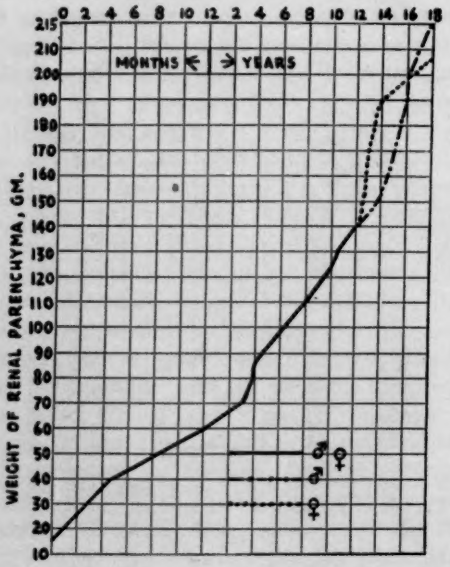

Chart 2.—Curve from which can be read the weight of the renal parenchyma in persons under 18 years of age.

always more cumbersome use of large tables of "surface areas," and the results are more reliable, especially with children.

I shall now briefly discuss the deduction of the formulas. In 1918 Addis [12] and in 1921 Austin, Stillman and Van Slyke [2] checked Ambard's two laws. This had already been done, though in a less complete manner, by Marshall and Davis in 1914. Often there has been confusion as to the meaning of the letters used in these formulas. In discussing the deduction of formulas, one must retain Ambard's abbreviations, the meaning of which is as follows: By *C* is meant the number of grams of urea per liter of urine, and by *Ur*, the number of grams of urea per liter of blood. By *V* is meant the number of

cubic centimeters of urine, and by D (debit), the number of grams of urea, which would have been secreted in twenty-four hours if during those twenty-four hours the diuresis had been of the same grade as the diuresis during the test period. This conversion at twenty-four hours is quite arbitrary. One might as well have chosen sixty minutes or another space of time. I thought it much simpler to calculate the minute volume (M), as American writers also have done. These writers use other letters and express the values for urea nitrogen in milligrams per hundred cubic centimeters (U, urine urea nitrogen in milligrams per hundred cubic centimeters; B, blood urea nitrogen in milligrams per hundred cubic centimeters; V, the number of cubic centimeters of urine per minute, and C, the urea clearance). It cannot be denied that the meanings of these letters created confusion, since the original abbreviations recommended by Ambard are still used in another part of the medical literature.

For the convenience of those who are accustomed to only one form of expression, I shall draw up the formulas for calculation of the indexes in both forms, expressing them, that is, in milligrams of urea nitrogen as well as in grams of urea. The figures for grams of urea should be multiplied by 46.5 to obtain the values in milligrams of urea nitrogen, so that one can simply replace $\frac{C}{Ur}$ by $\frac{U}{B}$. To designate minute volume, however, I advise that the letter M be used instead of the letter V, to avoid confusion with the aforementioned other significance of V in a part of the medical literature. The factor "surface area," used for correction of the urinary minute volume, is canceled and replaced by the factor N (weight [in grams] of the renal parenchyma). The abbreviations which I used in establishing the formulas for the iodosecretory index I shall mention later.

The aforementioned American writers came to the following conclusions:

The first law of Ambard, $K = \frac{D}{Ur^2}$, is correct if C remains constant. This condition was also made by Ambard. If C does not remain constant, distinction must be made between cases in which polyuria is present and those in which it is not. These improved laws were ascertained by experiments on dogs. Addis confirmed the results by experiments on rabbits. Ambard's first law must be taken as a particular instance (with C remaining constant) of more general laws.

The second law set up by Ambard proved to be wrong. It ran $k = D\sqrt{C}$, if Ur was constant. This law was valid only if $Ur\sqrt{D}$ was constant.

It is almost generally admitted that the criticism of Ambard's laws was to the purpose and that the new laws set up by the American investigators are correct. However, I looked for contradiction and found it, among other places, in the work of the French writers Chabanier and

Lobo-Onell.[8] They criticized all writers who dared contest Ambard's coefficient. It is interesting to know what judgment they passed on the aforementioned American investigations. They passed off the matter by saying that Van Slyke [22] revoked the results of the aforementioned correction of Ambard's laws. However, everybody reading Van Slyke's article sees at once that a revocation is out of the question. On the contrary, Van Slyke justly appreciated Ambard's work, but he stated the opinion that the results of his own examination were an improvement.

Since the deduction of the formulas by the American writers, I have tried to deduce two new formulas. The one new formula applies to cases in which polyuria is present; the other, to cases in which there is no polyuria. I have replaced the factor "surface area" by the weight of the renal parenchyma calculated in the aforementioned manner and based on observations at autopsy. It may be admitted that if, other things being equal, the diuresis is proportionately altered with the weight of the renal parenchyma the result will remain constant if the minute volume (M) is divided by the weight of the renal parenchyma (N). When the formula suitable for cases in which no polyuria is present is multiplied by 40 (for the iodosecretory index) or by 28 (for the ureosecretory index) and when the formula suitable for cases of polyuria is multiplied by 29 (for the iodosecretory index) or by 20 (for the ureosecretory index), the average result for persons with healthy kidneys is 100. If in a case in which the kidneys are altered by disease a result of (e. g.) 30 is found, one may conclude that about 30 per cent of the renal tissue is still working sufficiently. In contrast with some other formulas, one has at once, without additional calculations, the indication in every case as to what percentage of the renal tissue is still working sufficiently. From a great number of examinations in cases of healthy and diseased kidneys I was able to establish that the physiologic fluctuations lie between 60 and 140. Values under 60 point out a pathologic renal hypofunction, and the values above 140 point out a hyperfunction of the renal tissue. With the aid of a pocket slide rule, to be mentioned later, the results of these formulas can be read within one minute.

After making many experiments, it appeared to me advisable to use the "polyuria formula" when the minute volume (M) is larger than 2 cc., although the limit seems to be somewhat different for different persons. With minute volumes of less than 2 cc., which form the majority, I use this formula for cases in which polyuria is not present. The same limit is adopted by American investigators. I have drawn up the formulas in such a manner that when the minute volume is exactly equal to 2 cc. it is immaterial which formula is used.

Of course, there are cases in which these formulas cannot be applied. I have already pointed out the fact that in the presence of a relative

22. Van Slyke, D. D.: Presse méd. **35**:214, 1927.

oliguria with regard to urea the ureosecretory index is worthless, because the urea is secreted at its limit concentration (concentratio maxima). The existence of this relative oliguria cannot be excluded with certainty in many cases. In these cases reliable results are obtained by replacing the ureosecretory index by the iodosecretory index.

That there is a relation between the secretory functions of the kidney with regard to different substances appears from the fact that the limit concentrations of different substances are isomolecular. When a person has a maximal concentration of urea in the urine, e. g., 50 Gm. in 1,000 cubic centimeters, the limit concentration of this urine with regard to dextrose will be 150 Gm. The molecular weight of dextrose, which is 180, is three times the molecular weight of urea, which is 60. Substances with renal thresholds, like dextrose, are not suitable for examination of the renal function. The substance most suitable, as far as I know, for the purpose is iodine, because it has no renal threshold and its atomic weight is high (127). Supposing that with diseased kidneys the limit concentration for urea in the urine is 10 Gm. per liter; the limit concentration for iodine will be equal to 21.1 Gm. per liter, because $60:127 = 10:21.1$. Should a maximum dose of iodine be given, the concentration of iodine will remain far below 21 Gm. per liter. Chabanier admitted that it would be possible to obtain a constant value with either healthy or diseased kidneys if in Ambard's formula urea were replaced by iodine. Indeed, his example proved that this replacement was allowable. I applied it, with the same results. In order to obtain for this iodosecretory index the average result 100, the formula must be multiplied by another number than that used for the ureosecretory index. As a result of all these considerations, the following formulas were set up:

I. I. = iodosecretory index. Iu = mg. of iodine per hundred cubic centimeters of urine.

Ib = mg. of iodine per hundred cubic centimeters of blood.

U. I. = ureosecretory index. C = Gm. of urea per liter of urine. U = urea nitrogen in milligrams per hundred cubic centimeters of urine. Ur = Gm. of urea per liter of blood. B = urea nitrogen in milligrams per hundred cubic centimeters of blood.

N = weight (in grams) of the renal parenchyma.

M = minute volume in cubic centimeters. (Designated as V in a part of the literature.)

When $M < 2$, I. I. $= 40 \times \frac{Iu}{Ib} \times \sqrt{\frac{M}{N}}$

When $M > 2$, I. I. $= 29 \times \frac{Iu}{Ib} \times \frac{M}{\sqrt{N}}$

When $M < 2$, U. I. $= 28 \times \frac{C}{Ur} \times \sqrt{\frac{M}{N}} = 28 \times \frac{U}{B} \times \sqrt{\frac{M}{N}}$

When $M > 2$, U. I. $= 20 \times \frac{C}{Ur} \times \frac{M}{\sqrt{N}} = 20 \times \frac{U}{B} \times \frac{M}{\sqrt{N}}$

How the preparation should be made—and this is of the greatest importance for obtaining reliable results—and how the calculation can be done very quickly will be described in the following pages.

With healthy persons and also with patients not showing a relative oliguria with regard to urea, I was able to establish that the results agreed well when both the ureosecretory and the iodosecretory indexes are calculated for the same person. If there is a relative oliguria with regard to urea, only the iodosecretory index can give reliable information. Since the chemical test for the iodosecretory index takes less time, nobody will object to the thesis that as a routine method the determina-

TABLE 2.—*Determination of Both Indexes in the Same Examination* *

Patient	Examination	M	Iu, Mg. per 100 Cc.	Ib, Mg. per 100 Cc.	I. I.	C	Ur	U. I.
Miss v. N.	1st	0.8	380	8	110	24.4	0.36	108
	2d	1	402	9.3	110	26.6	0.45	107
Mrs. B.		0.5	300	8	68	23.9	0.436	70
Mr. V. A.		0.65	410	12	71	23.2	0.49	70
Miss A.		2.35	67.5	10.3	29	8.2	0.8	29
Mr. R.		2.1	45	10.5	17.4	7.6	1.07	19

* On the day after the first examination the patient (v. N.) was examined again (verifying examination).

TABLE 3.—*Influence of Oliguria on the Ureosecretory Index* *

Patient	M	Iu, Mg. per 100 Cc.	Ib, Mg. per 100 Cc.	I. I.	C	Ur	U. I.
Mr. V. S.	0.318	600	13.2	68	21	0.560	37
Miss W.	0.417	664	11.3	104	20.8	0.465	53

* Instances of low urinary secretion, in which the iodosecretory index only was reliable. In a clinical sense neither Mr. V. S. nor Miss W. suffered from a renal disease.

tion of the iodosecretory index is preferable to that of the ureosecretory index.

I shall first mention some instances which show that the iodosecretory index agrees well with the ureosecretory index when applied to the same person (table 2). In cases in which the agreement was less satisfactory clinical observations advocated the accuracy of the iodosecretory index.

In table 3 are noted the iodosecretory and the ureosecretory indexes of 2 patients, whose slight diuresis during the test period and whose high level of blood urea supported the possibility of a relative oliguria with regard to urea. The results for the iodosecretory index agree with the clinical picture, whereas the results for the ureosecretory index are at variance with it. That the bases on which the formulas rest are solid is clearly demonstrated by the often striking agreement of the

values for the iodosecretory and those for the ureosecretory indexes if a relative oliguria is excluded, notwithstanding quite different values for *M, Iu, Ib, C* and *Ur*. Table 4 refers to persons with healthy kidneys. In two successive examinations, the values for *Iu, Ib, C, Ur* and *M* sometimes differed greatly whereas the indexes agreed well. In these cases no polyuria was present. Table 5 shows that the existence of an "augmentation limit" must be admitted. For this I selected indexes established in the cases of persons with healthy kidneys who during the

TABLE 4.—*Results of Repeated Examinations at Short Intervals* *

Patient	Examination	M			
Mr. H.	1st	1.26	Iu = 260 mg./100 cc.	Ib = 11.8 mg./100 cc.	I. I. = 64
	2d	1.13	Iu = 226 mg./100 cc.	Ib = 10.3 mg./100 cc.	I. I. = 61
Miss H.	1st	0.97	C = 15.6	Ur = 0.29	U. I. = 96
	2d	0.38	C = 24.5	Ur = 0.275	U. I. = 99
Miss B.	1st	0.54	C = 22.6	Ur = 0.35	U. I. = 88
	2d	0.44	C = 17.3	Ur = 0.243	U. I. = 87
Mrs. B.	1st	1.55	C = 9.7	Ur = 0.324	U. I. = 70
	2d	0.46	C = 10.8	Ur = 0.189	U. I. = 73
Mrs. C.	1st	0.89	C = 15	Ur = 0.286	U. I. = 94
	2d	0.43	C = 20.28	Ur = 0.26	U. I. = 98

* This table shows the good agreement in the results when at an interval of a few days the examination of the same person was repeated. The subjects were persons with healthy kidneys, and polyuria was not present.

TABLE 5.—*Importance of Selection of Correct Formula in the Individual Case* *

Patient	Examination	C	Ur	M	N	UI Formula Intended for Patients with Polyuria	UI Formula Intended for Patients Without Polyuria
Miss D.	1st	4.5	0.35	4.5	225	77	51
	2d	2.8	0.285	5.9	225	77	34
Mr. L.	1st	5.8	0.365	4.15	260	82	56
	2d	6	0.24	2.6	260	81	70
Mrs. H.	1st	4.85	0.38	4.45	240	73	49
	2d	1.82	0.104	3.1	240	69	56

* This table shows that the clinician obtains a good agreement in the results of the first and in those of the second (verifying) examination only if he uses in the cases of polyuria the formula intended for them and not the formula intended for cases in which no polyuria is present. The patients were persons with healthy kidneys, and in all cases there was polyuria, i. e., M was larger than 2 cc.

test accidentally showed polyuria again and again. To compare them, I made calculations both with the formula set up for cases in which polyuria is present and with the formula set up for cases in which there is no polyuria. One sees at once how good the results are when the "polyuria formula" is used in cases of polyuria and how poor they are with use of the formula for cases in which there is no polyuria.

Table 6 conveys the same meaning. Applying a second test in these cases, I accidentally observed polyuria, which had not been present in the first examination. If the formula was applied in that second test as

in the first examination, the result was much poorer than when the "polyuria formula" was used.

Table 7 shows the results of successive examinations of patients suffering from serious Bright's disease. Miss W.'s condition was station-

TABLE 6.—*Comparative Results with Different Formulas* *

Patient	Examination	C	Ur	M	N	Results with Suitable U. I. Formula	Results with U. I. Formula for Patients Without Polyuria
Mr. E.	1st	17.6	0.416	1.11	244	80	
	2d	7.8	0.338	2.46	244	73	65
Mr. B.	1st	30.2	0.7	0.58	238	59	
	2d	5.98	0.416	3.1	238	57.5	46
Mrs. A.	1st	9.36	0.364	0.95	215	48	
	2d	4.45	0.341	2.5	215	44	40

* This table illustrates cases in which accidentally no polyuria was present in the first examination and in which in the second (verifying) examination polyuria was found. Like table 5, it points out the necessity of using the suitable formula.

TABLE 7.—*Results of Successive Examinations* *

Patient	Examination	C	Ur	M	N	U. I.
Miss W.	1st	14	1.74	0.67	224	12.4
	2d	17	1.58	0.445	224	13.5
	3d	9.5	1.02	0.45	224	11.7
Mr. H.	1st	11.4	1.75	1.8	239	16.2
	2d	13	1.89	0.79	239	11.2
Mr. V.	January 15	8.6	1.22	1.29	230	14.7
	May 22	7.75	1.16	1.17	230	13.4
	May 23	7.55	1.13	1	230	12.4
	December 17	7.5	1.32	1.17	230	11.4
	December 18	8.65	1.3	0.88	230	11.6

* This table shows the successive examinations of patients with renal disease. The first case shows that the poor condition of the kidneys remained constant. The 2 other cases show the progressive character of the course of the disease, which was also established clinically.

TABLE 8.—*Progressive Decline of Index Values in a Case of Bright's Disease* *

Patient	Date of Examination	C	Ur	M	N	U. I.
Mr. L.	8/8 36	19.4	0.54	1.02	233	67
	10/8 36	16.7	0.485	0.88	233	58
	7/8 37	10.4	0.442	1.08	233	45
	10/7 37	12.48	0.936	1.3	223	27.5
	11/7 37	9.88	0.884	1.33	233	23.5

* This table shows the steady decline of the ureosecretory indexes in 1 year in the case of a patient with serious Bright's disease.

ary, but Mr. H.'s and Mr. V.'s condition had grown worse on the dates of the successive clinical examinations, as is shown by the indexes.

Table 8 shows the results of examinations in the course of a year in a case of clinical progressive Bright's disease. In that year I determined the ureosecretory index five times. One sees the constant decline of the indexes.

A difficulty in applying these formulas is that some persons do not empty the bladder completely at the prescribed time. If one suspects retention of urine, a catheterization of the bladder can be applied. Male patients, however, dislike it sometimes to such an extent that they do not allow a second (verifying) examination, which, if possible, I make within a week after the first examination. Moreover, one sometimes is not sure whether the bladder has been completely emptied via the catheter. Many years ago, knowing the examinations in Utrecht of Verploegh and Hoogenhuyže, of which mention will be made later, I tried to make use of creatinine secretion to avoid this source of errors. Later I saw that other investigators also used this means. The fact that Behre and Benedict [23] suggested, that the actual creatinine is not responsible for the change in color, does not detract from the practical value of the creatinine test in this case. As far as I was able to ascertain, physicians had not yet arrived at a systematic application of the creatinine method, because this method takes too much time. It was recommended to make determinations of the level of creatinine in the urine with the aid of solutions of coloring matters which were of a different chemical composition from creatinine and for this reason of a somewhat different color. This difference in color was sufficient to make the determinations unreliable, as was proved by my experience. However, I have now succeeded in simplifying this complicated method so that quantitative determinations of the concentration of creatinine can be dispensed with. This method takes little time and yet produces reliable results, as the verifying test has shown. Only the use of a good colorimeter is required, e. g., the Duboscq type of colorimeter or a Zeiss-Ikon colorimeter. I make use of certain well known facts: first, the fact that the patient is nearly always able to empty the bladder more completely when the period of filling is somewhat prolonged than when it is relatively short, and second, that with the same retained quantity the error made in calculating the minute volume will be slighter, as the voided quantity will be larger. Suppose that the kidneys of a person secrete 300 cc. of urine in three hundred minutes. The quantity of urine voided is only 270 cc. The amount retained is 30 cc., and the minute volume seems to be $\frac{270}{300} = 0.9$ cc., whereas it is actually $\frac{300}{300} = 1$ cc. The error per minute volume is 0.1 cc. Further, suppose that some hours later the kidneys of the same person secrete 60 cc. of urine in the course of sixty minutes. The quantity of urine voided is only 30 cc. The amount retained is 30 cc., as in the former, longer period. The minute volume seems to be $\frac{30}{60} = 0.5$ cc., whereas it actually is $\frac{60}{60} = 1$ cc. The error per minute volume is 0.5 and so five times greater than with the same retained quantity in a five times longer

23. Behre, J. A., and Benedict, S. R.: J. Biol. Chem. **52**:11, 1922.

period of filling of the bladder. Of course, this longer period should not be extended too long, because complaints of dysuria will then arise. A period of about five to five and one-half hours has proved suitable in many cases for application of the creatinine method. The minute volume for this longer period (five and one-half hours), which may be called the preparation period, must be calculated by measuring the quantity of urine for this period. The minute volume for a shorter period (e. g., one hour), which may be called the test period, can be calculated by the application of the creatinine method without measuring the quantity of urine for this period. Incomplete emptying of the bladder after the short test period cannot render worthless the whole examination if emptying of the bladder after the preparation period has been complete.

The creatinine method is based on the fact that practically creatinine is secreted in equal quantities per unit of time in the same person and on the same day. This was demonstrated in 1905 by Verploegh and Hoogenhuyže at the laboratory of Pekelharing, Utrecht, and confirmed by Schaffer in 1908. Therefore, when two specimens of urine secreted by a patient in one day are compared, the minute volumes of these specimens will be inversely proportional to the concentrations of creatinine. I made use of the fact that these creatinine concentrations themselves are inversely proportional to the volumes which, with similarity of color, are read off on the colorimeter. In this way determination of the creatinine concentrations can be dispensed with, for the conclusion may be drawn that the minute volumes are directly proportional to the volumes read off on the colorimeter (with similarity of color). Standard solutions of coloring matters, which had to be prepared again and again and which, as has been mentioned, never were of exactly the same color as the creatinine, are then also dispensed with. In another way also I succeeded in dispensing with these standard solutions. I saw that the sliding glasses put on the market by the firm of Hellige for determining the concentrations of creatinine in blood are worthless for determining the concentrations of creatinine in urine. Therefore I made an experiment with the Zeiss-Ikon blood sugar meter. The colored glass of this apparatus shows the color of picramic acid (monoaminodinitrophenol) and this color agrees strikingly with the color of the urinary creatinine reaction. The agreement of the results with those obtained with the Duboscq type of colorimeter struck me. In this case the numbers of the Zeiss-Ikon colorimeter are used only as comparison values for the intensity of color. These numbers are directly proportional to the concentrations of creatinine. As has been stated, the latter values are inversely proportional to the minute volumes, whereas these minute volumes are directly proportional to the volumes read off on the Duboscq type of colorimeter. If M^1 is considered the minute volume for the preparation period, K^1 the concentration of creatinine in the urine for

this period, v^1 the volume read off on a Duboscq type colorimeter, and if one calls the same values for urine collected during the test period respectively M, K and v, the result is, as has been mentioned: $\frac{M}{M^1} = \frac{K^1}{K} = \frac{v}{v^1}$.

In a certain case, in which M^1 was 0.33, a laboratory assistant, using a Duboscq type of colorimeter, read off $v = 19.1$ and $v^1 = 20$, and I, without knowing these numbers, read off on a Zeiss-Ikon colorimeter $K = 320$ and $K^1 = 310$. In both readings the value for M was exactly the same, 0.318. It does not matter, therefore, which of the two methods is used.

I shall now mention a few instances to show how accurate is the result of the creatinine method.

Mrs. S., after the test period was past, voided 50 cc. of urine. With the aid of the creatinine method I estimated this value at 49.6 cc. Miss W., after the test period was past, voided 25 cc. of urine. With the

TABLE 9.—*Results With and Without Use of the Creatinine Method* *

Patient	Examination	C	Ur	N	M Without Creatinine Method	M with Creatinine Method	U. I. Without Creatinine Method	U. I. with Creatinine Method
Mr. S.	1st	8.2	1.5	236	3.26	1.41	24.8	11.9
	2d	8.2	1.45	236	1.12	1.45	11	12.4
Mrs. B.	1st	24	0.35	214	0.2	0.49	61	92
	2d	21	0.24	214	0.28	0.27	89	88

* This table shows that in these cases the results in repeated examinations agreed much better when the minute volume was determined with the aid of the creatinine method. For further explanation, see text.

aid of the creatinine method, I estimated this value at 25.02 cc. In the case of Mr. N., the values were 41.7 and 42 respectively.

It is astonishing what accurate results can be obtained with this simple colorimeter method.

In cases in which I suspected retention of urine at the beginning or at the end of the test period I often found on determination of the iodosecretory or of the ureosecretory index that the results showed more accurate agreement at the first and at the second (verifying) examination if the numbers obtained with the aid of the creatinine method were used than by direct measuring of the quantity of urine.

In table 9 it is seen that at the first examination of Mr. S. with the creatinine method I found a much lower value for M than by direct measuring without the creatinine method. At the second (verifying) examination of the same subject it was just the reverse, though in a less pronounced degree. The table shows that in calculation of the ureosecretory index the numbers agree well (11.9 and 12.4) if the results of the creatinine method are used. The difference of the results

obtained without applying the creatinine method (24.8 and 11) indicates unreliability. In this case the creatinine method appeared to be indispensable, because catheterization was not allowed by the patient. That the quantity of urine measured at the first examination was much larger than the quantity calculated by the creatinine method is due to the fact that at the beginning of this test the patient showed a retention of urine, whether only at the beginning or in a more marked degree at the beginning than at the end of the test period. At the verifying test the reverse was true.

The second patient, Mrs. B., showed at the first examination a greater retention at the beginning of the test period. That in this case too the results of the ureosecretory index calculated with the aid of the creatinine method were more reliable than the results obtained without applying the creatinine method is shown in table 9 (92 and 88 over against 61 and 89).

Considering these results, one might raise the question whether it is not advisable to apply this simple creatinine method in all cases for determining the diuresis during the test period, especially as the use of the catheter does not guarantee complete emptying of the bladder.

On applying ureteral catheterization for examining the renal function of each of the kidneys separately, one encounters the difficulty that a part of the urine often runs outside along the ureteral catheter into the bladder. With the creatinine method one can establish in a reliable way how much urine is secreted in a certain period by each of the kidneys separately. There exists an ingenious, though simple, calculation to establish (without this creatinine method) how much urine has been secreted by each of the kidneys in a certain period. The two methods can be combined for verifying purposes. When the ureteral catheters have been placed, the water brought into the bladder is allowed to run away through a bladder catheter. Everything depends on whether catheterization of the bladder is done completely, because if it is not done completely the method of calculation is worthless. Suppose that all the water has been removed from the bladder and that from this point of time, during a certain period, x cc. of urine runs outside along the right ureteral catheter into the bladder and y cc. of urine outside along the left ureteral catheter. When the test is finished, the bladder is again emptied with the catheter. Suppose, further, that the bladder catheter produces vb cc., then $vb = x + y$. When inside, the right ureteral catheter runs a volume of vr cc., and when inside, the left ureteral catheter runs a volume of vl cc. When, further, the number of grams of urea per cubic centimeter in the urine of the bladder is ub and this value for the urine secreted by the right kidney is ur and for that secreted by the left kidney is ul, then $(x \times ur) + (y \times ul) = vb \times ub$. There are now two comparisons, with two unknowns, with which x and y can be cal-

culated. In reality the right kidney has secreted $(vr + x)$ cc. and the left kidney $(vl + y)$ cc. I have described this method here also to demonstrate that examination with the aid of the creatinine method is to be preferred since one can never make quite sure whether the water and, later, the urine have been completely removed from the bladder.

If it is only a matter of comparing the function of the two kidneys, the chromocystoscopic and the pyelographic procedures introduced by Von Lichtenberg and Strick are sufficient. However, it is also of much importance to get an accurate view of the absolute function of a kidney, e. g., a kidney that will be left in the body after unilateral nephrectomy. In such cases the determination of the secretory index gives a reliable insight. It must therefore be combined with chromocystoscopic or pyelographic procedures. Each surgeon in his treatment of urologic patients is often faced with the question whether one kidney (the best of the two or a solitary kidney) functions sufficiently. Application of the aforementioned method can give the correct answer.

TABLE 10.—*Examination of Renal Function of a Dog* *

	C	Ur	M	N	U. I.
Before extirpation of one kidney...	12	0.3	0.21	40	88
Day after operation.....	5.1	0.505	1.27	40	51
Three days afterward.....	7.35	0.456	0.64	40	57

* This table shows the influence on the U. I. of extirpation of one kidney. The calculations have been made as if it were unknown that one kidney had been removed. (The extirpated kidney weighed 20 Gm., and so it was assumed that both kidneys weighed 40 Gm.).

In applying the secretory indexes, one should always take into consideration that the results of them never give one a right to judge of the anatomic extensiveness of lesions, because other parts of a partly diseased kidney may show compensatory hyperfunction. This general rule must not be overlooked. Compensatory hyperfunction of a healthy kidney often occurs when the other kidney is diseased or has been removed. Examining solitary kidneys, I repeatedly found an index agree with the functional ability of two healthy kidneys. When both kidneys function normally, the secretory index of one of the kidneys, on account of physiologic fluctuations, lies between 30 and 70, averaging 50. Of course the weight of the parenchyma of each of the kidneys must be taken into account. Thus, in one case I found for the function of one kidney (both kidneys appearing to be healthy) a ureosecretory index of 55 if I took into account the total parenchyma of both kidneys and a ureosecretory index of 94 if I took into account half the weight of the total renal parenchyma.

As a verifying test, I asked a surgeon to remove a kidney of a healthy dog. Before and after the nephrectomy I obtained the results given in table 10.

It is now possible to establish before nephrectomy the absolute function of the remaining kidney, independent of a relative oliguria with regard to urea, with the aid of the iodosecretory index and the creatinine method.

Reliable knowledge of the renal function may also be of great importance in the differential diagnosis of albuminuria and Bright's disease.

A SIMPLE METHOD OF DETERMINING THE CONTENT OF IODINE IN URINE AND BLOOD AFTER ADMINISTRATION OF AN IODINE SALT

The aforementioned advantages of replacing urea by iodine in the testing for renal function would not have any practical significance if there were not a simple method, suitable for the clinic, of determining the level of iodine in blood and in urine. Several methods for iodine determination were tried by me after the patients had taken different quantities of iodine salts.

First of all, I applied incineration methods, in which destruction of the organic substance was obtained by melting with a salt mixture. This method is founded on the classic principle which Rabourdin described in 1850. Many modifications of it have been recommended. After the incineration, the iodine content was determined by titrimetric, gravimetric or colorimetric means. If one wishes to determine the very small quantities of iodine which are normally present in the whole blood (6 to 13 micrograms per hundred cubic centimeters), the method of the Swiss chemist Fellenberg may be used. With this method even 0.1 microgram can be determined. For determination of the much larger quantities of iodine present in blood and urine after administration of, e. g., 4 Gm. of sodium iodide, this method, which involves intensive manipulation, is far too sensitive and therefore unsuitable. For more detailed particulars, I refer the reader to the article of Veil and Sturm.[24] After 4 Gm. of sodium iodide has been taken, there is in the blood about 7 to 12 mg. of iodine per hundred cubic centimeters, and in the urine, 30 to 700 mg. Incineration methods are suitable to determine these quantities, but they take much time. Besides, a part of the iodine sometimes evaporates at oxidation temperature. A method in which incineration is dispensed with is based on absorption of the alkali iodide by acetone. The acetone is distilled off. The iodide is oxidized with potassium permanganate into iodate. After this it is titrated with a thio-solution. This acetone method, which, at my request, a chemist applied in a number of cases, is so complicated that in my opinion it cannot be recommended for clinical use. For instance, ether extracts of the fat from the dry substance are needed after

24. Veil, W. H., and Sturm, A.: Deutsches Arch. f. klin. Med. **147**:166, 1925.

the acetone has been distilled off. Sometimes the so-called "salt error" may make it impossible for more than 50 per cent of the iodine to be shaken out if one does not take precautionary measures.

Therefore, I asked the firm of Hellige to make for the most used colorimeters a special glass slide, with which a content of 7 to 12 mg. of iodine per hundred cubic centimeters in solutions (blood, urine) can be determined as accurately as possible. When urine was diluted so that the concentration of iodine in the urine reached about the level of that in blood, one glass slide, which had a measuring capacity for iodine of 7 to 12 mg. per hundred cubic centimeters, was sufficient for both blood and urine. This new iodine slide containing two colors is one of the best of the whole series of slides which this firm puts on the market. The colors of the extracts agree well with those of the glass slides. Besides, the firm has succeeded in extending the measuring capacity from 7 to 25 mg. of iodine per hundred cubic centimeters of solution. The execution of this method does not cause any difficulties and takes little time. It will be described later. As regards accuracy, this simple colorimetric method for determination of the concentration of iodine in blood and urine is undoubtedly inferior to the aforementioned elaborate acetone and incineration methods, but for clinical purposes it appears to be sufficiently accurate. The results obtained with this simple method are so encouraging that I thought it unnecessary to wait with the publication.

Chabanier [25] rightly pointed out the fact that the blood caught on sodium oxalate (or sodium fluoride) must be centrifuged at once to prevent diffusion of iodine in the dying erythrocytes. I have often seen evidence that this advice is correct. When I centrifuged a part of the blood at once and waited for spontaneous separation of the oxalated plasma in the other part, I found by comparison that the erythrocytes can hold 25 to 30 per cent of the plasma iodine. To extract this requires more complicated measures than to prevent diffusion by centrifuging the oxalated blood at once.

The very good agreement of the iodosecretory index and the ureosecretory index in many cases pleads in favor of this simple iodine determination. If the agreement is less satisfactory, the iodosecretory index matches better with the clinical symptoms than the ureosecretory index. Instances of this are noted in table 2.

Secretion curves for iodine after ingestion of 4 Gm. of sodium iodide have shown that the method described is reliable. Only then is it practically certain that during the test period fluctuations of the concentration of iodine in blood and urine remain as slight as possible. This is of very great importance for determination of the secretory indexes.

25. Chabanier, H.: Compt. rend. Soc. de biol. **78**:445, 1915.

Iodine determinations on blood cannot replace the urea determinations. As has been mentioned, one meets with cases in which, on account of hyperthyroidism or idiosyncrasy to iodine, determination of the iodosecretory index cannot be made. Determination of the ureosecretory index should in that case be preferred.

As to the method of the urea determination, it must be admitted that there are many reliable ones, but often they are needlessly cumbersome for the clinic, e. g., the xanthydrol method, the urease method and the mercury method. The more simple hypobromite method, which gives sufficiently correct results for the clinic, as was demonstrated by Chabanier, Becher, Schmid and Philiwert, has the disadvantage that close attention must always be paid to the air-tight fitting of the rubber bags or rubber stoppers when the tube is filled with fluid and gas. Small leaks may have existed for a long time before being detected. From the clinical laboratories of America the hypobromite methods have been largely discarded, and the urease methods have come into vogue. Newer methods can be compared with film stars. It is for both easier to get in than to get out through the large door. Dodson [26] stated justly in an American journal for technicians that the urease methods are time consuming and cumbersome, especially in routine work, in which many determinations are made daily. Dodson described an important simplification, but I pointed out that even with this good improvement the urease methods have many disadvantages as compared with a hypobromite method improved by means of a new ureometer.[27] In the same article the disadvantages of the old hypobromite methods are pointed out. I have tried to construct this simple ureometer for the hypobromite method in such a manner that the difficulties inherent in the other models are removed. The new ureometer possesses the following advantages: 1. Absence of either rubber bags or rubber stoppers. 2. Enclosure of the involved gas in a purely physical way (atmospheric pressure and salt solution of high specific gravity). This assures reliable results. 3. Simple construction, so that the price is low. 4. Strong construction, so that there is little danger of breaking it.

In the very rare cases in which venipuncture is impossible, the formulas must be calculated by the more complicated micromethods. Only in the hands of chemists or physicians of long chemical training do these micromethods give reliable results. This holds especially with regard to the iodine micromethods.

The calculation of secretory indexes can be done quickly with the aid of certain curves, which, at my request, a mathematician has drawn. However, slide rules appeared to me more practical. As the slide rules

26. Dodson, R. V.: Am. J. M. Technol. **6**:58, 1940.

27. Peters, J. T.: Am. J. M. Technol. **6**:160, 1940. (See also note at end of this article.)

put on the market were less suitable for these calculations and in general for the calculations with which a physician has to deal, I have designed a new pocket model. The attainable accuracy is 0.5 per cent, so that it is certainly sufficient for the purpose aimed at.[28]

After making many experiments, I arrived at the following directions regarding the period of preparation, the procedures for determination of the iodosecretory or the ureosecretory indexes and the determination of diuresis with the aid of the creatinine method. It is needless to say that these directions can be modified and adapted to circumstances in many ways. However, the modifications must not be of such extent that the purpose of the preparation period is missed, namely, to avoid as much as possible great fluctuations of the iodine content or of the urea content of the blood and urine during the period of examination.

For determination of both indexes I advise a period of preparation of five and one-half hours. As to the iodosecretory index, one should first make sure that there are no contraindications to the use of iodine (hyperthyroidism, idiosyncrasy to iodine, etc.). When there are none, the use of medicine should be avoided on the day of the test to prevent a possible disturbance of the color reactions in the iodine determination. Especially should acids or medicine from which acids are liberated be avoided. I observed this in the case of a patient who in ordinary circumstances tolerated sodium iodide well. One day, on account of a rheumatic complaint, he had taken some sodium salicylate shortly before taking the sodium iodide. Nausea and vomiting followed. Probably salicylic acid from the sodium salicylate liberated iodine from the iodides. This iodine may irritate the mucous membrane of the stomach by dilating the vessels and increasing the secretion. Care should be taken that no chloroform (to combat growth of bacteria) is added to the urine, because in this method chloroform is used as a means of extraction for iodine.

At the beginning of the preparation period the patient should urinate, trying to empty the bladder completely. The time of urinating should be noted accurately and the urine should be discarded. At intervals of half an hour he should take 0.5 Gm. of sodium iodide (e. g., two tablets of 250 mg. each dissolved in 15 cc. of water) until eight such doses (4 Gm.) have been taken. A half or whole peppermint tablet may be added to each dose to mask the flavor. If by mistake or on account of vomiting only 3 Gm. has been taken, the test can still be carried out, for I have observed that after ingestion of 3 Gm. the color reaction is sufficiently strong to finish the test. However, if more than 1 Gm. is vomited it is advisable to repeat the test, for the secretion by the kidneys forms only a part of the iodine secretion. The iodine is removed in many ways. If necessary, defecation should

28. See note at end of article.

precede the preparation period, for defecation during the test is to be avoided on account of a possible loss of urine. Within the first two hours of this preparation period a meal may be taken with (at most) 50 Gm. of meat or fish and (at most) 100 cc. of fluid. (Neither coffee nor tea should be taken, on account of the diuretic action of these substances.) When 4 Gm. of sodium iodide in doses of 0.5 Gm. at intervals of half an hour has been taken, one should wait two hours before beginning the test proper. However, the patient should continue to drink 15 cc. of water every half hour in order to avoid as much as possible greater fluctuations in diuresis during the test proper.

Two hours after the last dose of sodium iodide has been taken, the test proper begins. At this time the patient should urinate, trying to empty the bladder completely. The time should be noted accurately, and the urine should be kept only if one intends to apply the creatinine method to determine the diuresis. Should the patient be unable to retain the urine during five and one-half hours, he is allowed to urinate more than once during the preparation period. The specimens are accurately collected and are added to and mixed up with the specimen taken at the beginning of the test period. Half an hour after the beginning blood is taken, and half an hour later the patient urinates again, trying to empty the bladder completely. This urine is always kept and measured. The time of urinating at the beginning and at the end of the test period is accurately noted. If the creatinine method is not used, the specimen taken at the beginning of the test period is to be discarded.

1. *Determination of the Weight of the Renal Parenchyma.*—Note the body height in centimeters, indicated as L. For patients over 18 years of age, the weight of the renal parenchyma is indicated as $N = 1.4 \times L$. For patients under 18 years of age, the weight of the renal parenchyma can be read from chart 2.

2. *Determination of the Minute Volumes.*—M^1 is assumed to be the minute volume of the urine voided during the preparation period, and M, the minute volume of urine voided during the test period.

a. Without the Creatinine Method: Suppose that the test period lasts q minutes. After this the patient voids p cc. of urine. The minute volume for the test period is then expressed as $M = \frac{p}{q}$ cc.

b. With the Creatinine Method: The preparation period lasts s minutes. After this the patient voids r cc. of urine. The minute volume for the preparation period is: $M^1 = \frac{r}{s}$ cc.

I have called the minute volume for the test period M, the concentration of creatinine in the urine for the same period K and the volume of the urine, read with similarity of color in a Duboscq type of colorimeter, v. The same values for the urine of the preparation period

I have called: M^1, K^1 and v^1. When using the Zeiss-Ikon colorimeter (constructed for determination of the level of blood sugar), one calculates: $M = \frac{K^1 \times M^1}{K}$. When using a Duboscq type of colorimeter, one calculates: $M = \frac{v \times M^1}{v^1}$.

3. *Determination of the Iodosecretory Index (I. I.).*—a. Examination of the Urine: Measure 0.5 cc. of urine and place it in a measuring glass, in which previously 10 cc. of chloroform has been put. Add 3 cc. of 4 per cent potassium nitrite solution and 3 cc. of normal hydrochloric acid. Shake. Filter the colored chloroform solution through a dry filter in the tube or wedge of the colorimeter, in which the aforementioned "iodine sliding glass" has been placed. Various models of colorimeters can be used with different models of iodine sliding glasses. When, with similarity of color, one reads from a table (accompanying the sliding glass): p mg. of iodine in 100 cc. of chloroform, then the urine contains: $\frac{200 \times p}{10} = 20 \times p$ mg. of iodine per hundred cubic centimeters $= Iu$. If the color turns dark violet, add 10 cc. more of chloroform, in order to make the reading easier. Then the denominator 10 in the calculation should be replaced by 20.

b. Examination of the Blood: Twenty-eight to 30 cc. of oxalated blood is required. By means of a thicker venipuncture needle blood is allowed to run quickly into a glass cylinder on sodium oxalate. After thorough shaking, it is poured at once into two or four centrifugal tubes of such measurements that one has at one's disposal after centrifuging 15 cc. of oxalated plasma. Centrifuge as soon as possible. (A centrifugal tube filled with 15 cc. of oxalated blood produces 8 or 10 cc. of oxalated plasma when centrifuged in a strong centrifuge for ten minutes.) Add to 15 cc. of oxalated plasma an equal part of 20 per cent trichloroacetic acid. Shake thoroughly. Pour this mixture into a large filter; measure off 20 cc. of the filtrate (containing 10 cc. of plasma). This 20 cc. of filtrate is placed in a measuring glass into which previously 10 cc. of chloroform has been put. Add 3 cc. of 4 per cent potassium nitrite solution and 3 cc. of normal hydrochloric acid. Shake. Filter the colored chloroform solution through a dry filter in the wedge or tube of the colorimeter, in which the aforementioned iodine sliding glass has been placed. When, with similarity of color, one reads from the accompanying table q mg. of iodine in 100 cc. of chloroform then the blood contains $\frac{10 \times q}{10} = q$ mg. of iodine per hundred cubic centimeters $= Ib$.

4. *Determinations of the Ureosecretory Index.*—a. Urine Test (with the aforementioned ureometer): Suppose that 0.5 cc. of urine has been diluted with water to 50 cc. and of this diluted urine 10 cc. has been used for the test as described in the instructions accompanying

each ureometer and also in an article previously published.[27] When one reads on the ureometer a cc. of nitrogen, then the urine contains $2.7 \times \frac{a}{0.1}$ Gm. of urea per liter $= C$, or $12.6 \times a$ milligrams of urea nitrogen in 100 cc. of urine $= U$.

(The albumin should be removed from albuminous urine before the test is made.)

b. Blood Test (with the aforementioned ureometer): About 15 cc. of oxalated blood is recommended, although the ureometer can be used with much less blood. Add to it, in a glass cylinder, equal parts of 20 per cent trichloroacetic acid. Shake thoroughly. Pour this mixture into a filter. As one wishes to use 5 cc. of blood, 10 cc. of the filtrate should be mixed with the bromite solution in the ureometer according to the elaborate directions accompanying the apparatus. When one reads a cc. of nitrogen, the blood contains: $2.7 \times \frac{a}{5}$ Gm. of urea per liter $= Ur$, or $25 \times a$ milligrams of urea nitrogen in 100 cc. of blood $= B$.

If there was less than 10 cc. of filtrate, also half the quantity of the filtrate will consist of blood, and the number expressing this half replaces 5 in this comparison.

With these and the aforementioned data the formulas described can be easily calculated.

Withdrawal of 15 cc. of blood for the determination of the ureosecretory index or of 30 cc. of blood for the determination of the iodosecretory index is not harmful for patients who are not weakened by serious complications. In my experience with numerous patients, I never saw any harm done. Osgood [29] calculated that after withdrawal of 50 cc. of blood in ten days, *even if no regeneration of blood occurs,* but only the blood volume returns to its previous value, the lowering of the hemoglobin percentage is less than the normal daily variation.

As to the preparing of oxalated blood for these examinations, I recommend Osgood's method of making a stock of tubes, each containing 2 mg. of dry powdered sodium oxalate per cubic centimeter of blood. For example, in the tubes for 15 or 30 cc. of blood, one measures, with a buret, 1.5 or 3 cc. respectively of a 2 per cent solution of sodium oxalate and evaporates the water in an oven.

The protein-free filtrate should always be prepared as soon as possible after the withdrawal of the blood. It will keep well for twelve hours if placed directly in an ice box. For the urea determination, the preparation of the protein-free filtrate with trichloroacetic acid may be replaced by the tungstic acid method, but the latter is more cumbersome and is no better for this special case.

29. Osgood,[21] p. 461.

It is not advisable to subtract the ammonia nitrogen from the urea nitrogen of the urine (as has often been recommended), because ammonia is formed from urea in the kidney.

To detect errors in the technical execution, it is recommended to take two specimens of urine at the same test and to repeat the test if the results disagree more than 30 per cent. However, by this method an error is not detected if it is present only in calculation of the blood urea nitrogen. Therefore a repetition of the test within a week seems to me better.

If one wishes to judge at the same time of the quantity of blood urea with a nitrogen-deficient diet, meat, fish, eggs and milk should be forbidden on the day of examination. The same holds when a relative oliguria with regard to urea is suspected and there is no opportunity to determine the iodosecretory index, so that determination of the ureosecretory index is the only test left. In such cases the supply of fluid should be heightened a little. When there is no reason or no opportunity to apply the creatinine method for measuring the diuresis, then, of course, the period of preparation can be shorter, etc.

JUDGMENT OF THE RESULTS OF THESE TESTS

Both indexes, whose object is to determine the efficiency of the kidney tissue in the removal of an iodine salt or urea from the blood, can give only momentary pictures of the renal function. These momentary pictures like, e. g., roentgenograms of the kidneys, should be compared with the results of anamnestic, clinical, physical, chemical and microscopic examinations.

The slide rules, ureometers, slide glasses and colorimeters mentioned in this article are to be distributed by Eugene Baehr and Sons, 251 Fourth Avenue, New York.

AN EXPERIMENTAL STUDY OF RECIPROCATING RHYTHM

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NEW YORK

As evolution proceeded, the muscular connection between auricles and ventricles gradually diminished. In the piscine heart, junctional fibers are present and conduct equally well in either direction at the whole circumference of the auriculoventricular border. In the amphibian heart, some parts of the junctional tissue conduct better than others; in some sections, reversed, or ventriculoauricular, conduction is even more rapid than normal, or auriculoventricular, conduction. Differences in conductivity are even more pronounced in the reptilian heart, and a reduction in the amount of conduction tissue has occurred.[1] Differentiation and specialization are still more evident in the avian heart, and in the mammalian heart the relatively small auriculoventricular conduction system furnishes the only morphologic connection between auricles and ventricles. Thus, functional differentiation may be assumed to have preceded anatomic alteration and reduction.

A second communication between auricles and ventricles in man has been suggested as an atavistic, inherited anomaly occurring on rare occasions. Since each phylogenetic stage is repeated in ontogenesis, such observations become comprehensible.[2] This second junction between auricles and ventricles has been assumed by virtue of an analysis of certain peculiar electrocardiograms recorded in man; anatomic confirmation has not been secured.

From the New York Medical College and the Flower and Fifth Avenue Hospitals. Aided by a grant from the Ella Sachs Plotz Foundation.

1. von Skramlik, E.: Ueber die anatomische Beschaffenheit der Ueberleitungsgebilde des Kaltblüterherzens, Ztschr. f. d. ges. exper. Med. **14**:246, 1921; Ueber die Beziehungen zwischen der normalen und rücklaufigen Erregungsleitung beim Froschherzen, Arch. f. d. ges. Physiol. **184**:1, 1920.

2. Kent, A. F. S.: Researches on the Structure and Function of the Mammalian Heart, J. Physiol. **14**:233, 1893. Holzmann, M., and Scherf, D.: Ueber Elektrokardiogramme mit verkürzter Vorhof-Kammer-Distanz und positiven P-Zacken, Ztschr. f. klin. Med. **121**:404, 1932. Wolferth, C. C., and Wood, F. C.: The Mechanism of Production of Short P-R Intervals and Prolonged QRS Complexes in Patients with Presumably Undamaged Hearts: Hypothesis of an Accessory Pathway of Auriculo-Ventricular Conduction (Bundle of Kent), Am. Heart J. **8**:297, 1933. Scherf, D., and Schönbrunner, E.: Beiträge zum Problem der verkürzten Vorhofkammerleitung, Ztschr. f. klin. Med. **128**:750, 1935.

One of the outstanding problems of auriculoventricular conduction still subject to discussion is the function of, and conductivity in, the auriculoventricular node. Its position in the center of the heart, almost inaccessible to experimental investigation, is one of the important reasons for the meager knowledge regarding its physiology. The present experimental study was undertaken with the purpose of investigating a remarkable disturbance of cardiac action in which mainly the auriculoventricular node itself seems to be involved.

After the application of a series of electrical stimuli to the heart of the frog or the fish, Mines noticed a peculiar disturbance of cardiac rhythm.[3] Auricles and ventricles contracted alternately, and it could be shown that a wave was conducted in a circle going from the auricles to the ventricles and then back from the ventricles to the auricles and so forth. This disturbance was explained by the assumption of a slight difference in the length of the refractory period in certain parts of the junctional tissue between auricles and ventricles. The stimulus thus traveled in one part of this tissue from the auricle to the ventricle and in another from the ventricle to the auricle.

An observation of a similar disturbance in man was reported and correctly interpreted by White.[4] A series of additional observations followed.[5] Similar but dubious cases may be found in the literature prior to the invention of the electrocardiograph.[6] In all these cases an auriculoventricular rhythm existed. Sometimes, without any other

3. Mines, G. R.: On Dynamic Equilibrium in the Heart, J. Physiol. **46**:349, 1913.

4. White, P. D.: A Study of Atrioventricular Rhythm Following Auricular Flutter, Arch. Int. Med. **16**:517 (Oct.) 1915; The Bigeminal Pulse in Atrioventricular Rhythm, ibid. **28**:213 (Aug.) 1921.

5. (*a*) Bishop, L. F.: Specific Action of Atropin in Relieving Certain Irregularities of Heart Beat, J. A. M. A. **77**:31 (July 2) 1921. (*b*) Blumgart, H. L., and Gargill, S. L.: Reciprocal Beating of the Heart, Am. Heart J. **5**:424, 1930. (*c*) Cutts, F. B.: Reciprocal Rhythm in a Patient with Congenital Heart Disease, ibid. **14**:717, 1937. (*d*) Dock, W.: The Reciprocal Rhythm, Arch. Int. Med. **41**:745 (May) 1928. (*e*) Drury, A. N.: Paroxysmal Tachycardia of A-V Nodal Origin, Exhibiting Retrograde Heart Block and Reciprocal Rhythm, Heart **11**:405, 1924. (*f*) Gallavardin, L., and Gravier, L.: Bradycardie nodale permanente; étude du rythme atrio-ventriculaire, Arch. d. mal. du cœur **14**:71, 1921. (*g*) Gravier, L.; Froment, R., and Guiran, J. B.: Brady-arythmie sinusale avec automatisme ventriculaire permanent et "reciprocal rhythm," ibid. **32**:622, 1939. (*h*) Reid, W. D.: Permanent Bradycardia Following Diphtheria, Am. Heart J. **5**:524, 1930. (*i*) Samojloff, A., and Tschernoff, A.: Reziproker Herzrhythmus beim Menschen, Ztschr. f. d. ges. exper. Med. **71**:768, 1930.

6. Wenckebach, K. F.: Beiträge zur Kenntnis der menschlichen Herztätigkeit: V. Ueber Dissoziation der Tätigkeit beider Vorkammern, Arch. f. Physiol., 1906, p. 297. Muskens, L. J. J.: Genesis of the Alternating Pulse. J. Physiol. **36**:104, 1907.

obvious cause, during carotid pressure or during digitalis therapy backward conduction occurred from the auriculoventricular node to the auricle; occasionally this was delayed to such a degree that the auricles

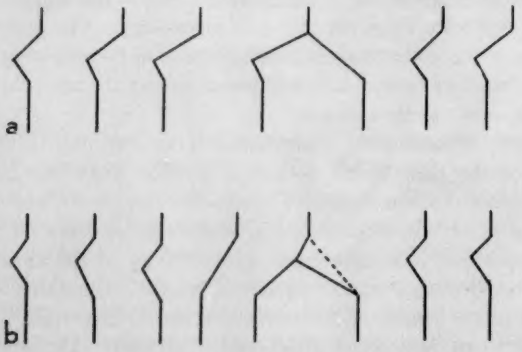

Fig. 1.—(*a*) The mechanism of a reciprocating beat during auriculoventricular rhythm with gradually prolonged reversed conduction. (*b*) The mechanism of a return extrasystole after a reversely conducted ventricular extrasystole during auriculoventricular rhythm.

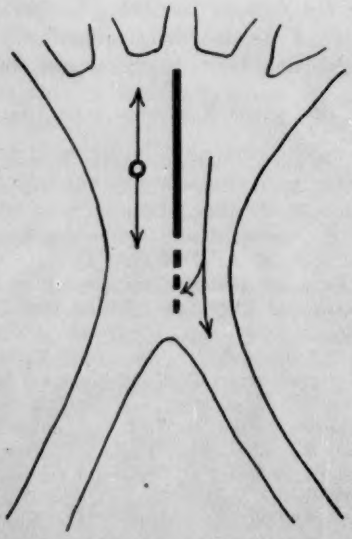

Fig. 2.—The drawing indicates the longitudinal functional dissociation in the auriculoventricular node (heavy line in the center) during auriculoventricular rhythm. The part of the conduction system just above the bifurcation may be employed by both the return extrasystole and the preceding, initiating beat.

contracted considerably later than the ventricles and an inverted P wave appeared some time after the QRS complex. This was followed by a new ventricular systole. It was assumed that the stimulus originating in the auriculoventricular node was reversely conducted from the node to the auricle and then returned once again to the ventricle, thus causing a second ventricular systole (reciprocating beat). The schematic drawings in figures 1 *a* and 2 explain this mechanism. In the cases reported, the auriculoventricular rhythm was usually the result of a lesion in the sinus node. However, *single* reciprocating (or reciprocal) beats occurred in the majority of the clinical observations; whereas Mines described a *continuous* reciprocating rhythm. In only 1 case [51] were tracings taken, which suggest, but do not prove, the possibility of a short period of reciprocating rhythm.

There have been no experimental studies concerning this arrhythmia, but a similar disturbance has been described in dogs; an auriculoventricular rhythm appeared after a clamp was applied to the sinus node.[7] Electrical stimulation was then employed to produce a series of ventricular extrasystoles. Usually they were conducted backward to the auricle. Frequently the last extrasystole of a series which was conducted backward to the auricle "returned" and again reached the ventricle; thus a premature ventricular systole followed (fig. 1 *b*). (The new premature abnormal beat has been called "return extrasystole" [reciprocating beat]). Some observations indicated that the last ventricular extrasystole reversely conducted turned back in the auriculoventricular node and once more reached the ventricle. The observation that the PR interval of the return extrasystole became shorter as the length of the preceding RP interval became longer seemed to suggest that at least a part of the pathway was used twice by the stimulus.

EXPERIMENTAL OBSERVATIONS

The experiments were performed on dogs whose hearts had been exposed by opening the chest wall and the pericardium. Both vagus nerves were cut in the neck. Ether or pentobarbital sodium anesthesia was used. An auriculoventricular rhythm was induced by compressing the sinus node with three or more clamps, as well as by clamping the arteries of the sinus node. In some experiments the action of the sinus node was inhibited by cooling. Any one who has tried to eliminate the action of the sinus node experimentally knows how difficult this task may be, since aberrant parts of it or variations in its position are not uncommon. For the purpose of these experiments it was important to secure that form of auriculoventricular rhythm in which the auricles contracted *after* the ventricles. This was difficult; frequently the more common type appeared, and all chambers contracted simultaneously. In some experiments the injection of 0.01 to 0.02 Gm.

7. Scherf, D., and Shookhoff, C.: Experimentelle Untersuchungen über die "Umkehr-Extrasystole" (Reciprocating Beat), Wien. Arch. f. inn. Med. **12**:501, 1926.

of quinidine sulfate was necessary to prevent ventricular fibrillation occurring after application of rapid electric stimuli to the ventricles. Often this amount of quinidine impaired the reversed conduction from the auriculoventricular node to the auricle sufficiently to give the form of auriculoventricular rhythm desired, that is, the P wave following the QRS complex. If this form of rhythm was present, stimulation of the left vagus nerve with a weak faradic current frequently produced prolongation of the RP interval.

In 11 of 28 experiments, return extrasystoles were observed. The experiments may be divided into two groups. In one, the abnormal rhythm was evoked by fatiguing the conduction system with many extrasystoles following each other at short intervals. In the other, the abnormal rhythm appeared during stimulation of the left vagus nerve with a weak faradic current.

In figures 3 *A* and *B* the application of induction shock stimuli is marked at the top by an electrical signal. Then follow the contractions of the right auricle and the right ventricle recorded by mechanical tracings. The time record in these tracings amounts to 0.02 second. In all others it is 0.04 second.

The auriculoventricular rhythm recorded in figure 3 *A* appeared after the sinus node was clamped. The mechanical tracings show that the auricles contracted after the ventricles, and in the electrocardiogram an inverted P wave is visible after the QRS complex. Observation for a period of five minutes did not reveal any change of rate or rhythm. At this time auricular extrasystoles were produced, and eight of them were conducted to the ventricle. Immediately thereafter, the auriculoventricular rhythm reappeared. Prior to the stimulation of the auricle, the P wave was visible 0.04 second after the beginning of the initial complex; in the first postextrasystolic beat after the extrasystoles it appeared after 0.10 second. It was followed after 0.11 second by a slightly aberrant premature beat (return extrasystole). Then an alteration of the reversed conduction from the auriculoventricular node to the auricle ensued because one RP interval measuring about 0.05 second alternated with the one following which measured approximately 0.09 second. Every beat with the longer RP interval was followed by a return extrasystole. After the appearance of six to eight of these extrasystoles the alternation of the reversed conduction stopped, and the auriculoventricular rhythm showed constantly the same short RP interval as before the extrasystoles. A new series of auricular extrasystoles was released five times, and the same disturbance always followed. The diagnosis of a return extrasystole in this tracing was based on the fact that it appeared only after those artificial auricular extrasystoles which caused a prolongation of the RP interval and disappeared as soon as this interval became shorter.

In another experiment (fig. 3 *B*), clamping the sinus node caused an auriculoventricular rhythm in which the auricles and ventricles contracted practically at the same time (first beat from the left). Four induction shocks caused three ventricular extrasystoles; one of them was conducted in a retrograde manner to the auricle as shown by the suspension tracing. In the first postextrasystolic auriculoventricular beat the auricles contracted 0.02 second after the ventricles; in the next auriculoventricular beat the auricles were activated 0.08 second later

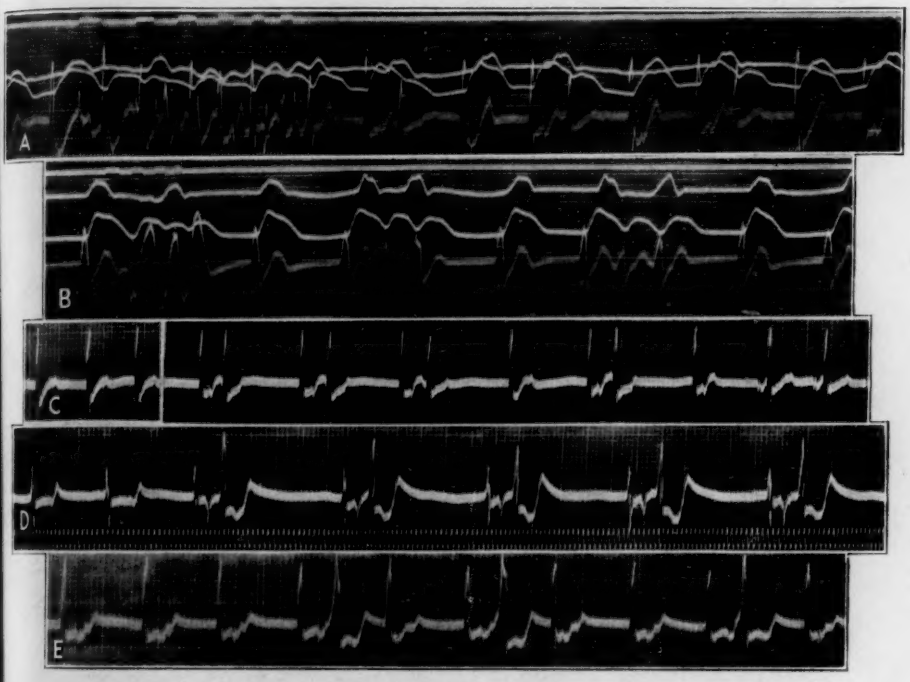

Fig. 3.—*A*, the first two beats show an auriculoventricular rhythm; after a series of auricular extrasystoles an alternation of the reversed conduction from the node to the auricle follows. A return extrasystole appears after every beat with a prolonged RP interval. *B*, the first beat shows an auriculoventricular rhythm. After three ventricular extrasystoles an alternation of the length of the RP interval appears with two return extrasystoles after the beat with the longer RP interval. *C*, the first three complexes show an auriculoventricular rhythm with an inverted P wave, just visible after the QRS complex. Faradic stimulation of the left vagus nerve causes a prolongation of the RP interval and initiates return extrasystoles. *D*, bigeminal rhythm due to return extrasystoles during stimulation of the vagus nerve. *E*, "interpolated" return extrasystoles without disturbance of the auriculoventricular rhythm.

than the ventricles, although not late enough to cause a clearcut P wave after the initial complex. The mechanical tracing, however, permitted measurement of the RP interval. In the following cycles one sees again the continuous alternation of a short and a long RP distance in the auriculoventricular beats as in figure 3 *A*. Here again, a return extrasystole appeared after every beat with a longer RP distance. The PR interval of this beat was 0.24 second long. In this experiment, however, the return extrasystole was again conducted backward to the auricle with a conduction time of 0.14 second and was followed by a second return extrasystole whose auriculoventricular conduction time was 0.13 second. The alternation in length of the RP interval as well as the return extrasystole disappeared after four or five cycles. Here also, renewed stimulation of the ventricle, repeated three times, initiated the same picture. The diagnosis of a return extrasystole not only was confirmed by the analysis of the electrocardiogram and the mechanogram but was substantiated by the fact that the peculiar trigeminal groups invariably appeared if the RP interval of the auriculoventricular beats was sufficiently prolonged but appeared at no other time.

These experiments showed that under certain conditions a bigeminal and a trigeminal rhythm may be due to return extrasystoles.

In the second group of experiments an auriculoventricular rhythm was evoked by compressing the sinus node and by clamping its arteries. If after this procedure an auriculoventricular rhythm appeared in which the excitation of the auricle occurred immediately following the excitation of the ventricle, the left vagus nerve was stimulated with a weak faradic current. In this manner one usually succeeds in producing a reversed block between the auriculoventricular node and the auricle, as demonstrated by the appearance of an RP interval.[8]

The first three beats in figure 3 *C* show a regular auriculoventricular rhythm with an inverted P wave just visible after the initial ventricular complex. Stimulation of the left vagus nerve prolonged the RP distance to about 0.12 second. The first three auriculoventricular beats and the fith beat are followed by a return extrasystole with a PR interval of about 0.12 second. The RP interval of the fourth beat is shorter, and therefore it is not followed by a return extrasystole. At the end of the tracing two sinus beats with inverted P waves and a PR interval of 0.11 second appear; this indicates that the clamps did not succeed in completely destroying the action of the sinus node. The "escape" of sinus beats during the auriculoventricular rhythm induced by cooling the sinus node has been observed and discussed by Lewis and White.[9]

8. Lewis, T.: The Effect of Vagal Stimulation upon Atrioventricular Rhythm, Heart **5**:247, 1914.

9. Lewis, T., and White, P. D.: The Effects of Premature Contractions in Vagotomised Dogs, with Especial Reference to Atrioventricular Rhythm, Heart **5**:335, 1914.

Repeated stimulation of the left vagus nerve always resulted in the same disturbance. As in the previous experiment (fig. 3 *B*), the abnormal beats appeared exclusively and regularly as soon as the RP interval was sufficiently prolonged.

In another experiment (fig. 3 *D*), the auriculoventricular rhythm appeared after the artery of the sinus node was clamped and after the application of three clamps to the sinus node itself. Auricles and ventricles contracted simultaneously. After the first two beats the left vagus nerve was stimulated. Immediately a deep inverted P wave appeared, about 0.11 second after the initial complex. After 0.12 second it was followed by a slightly aberrant return extrasystole. It is interesting to note that this return extrasystole appeared at a time when the T wave of the auriculoventricular beat had not as yet begun. In this experiment, as well as in others, continuous bigeminal rhythm appeared whenever the vagus nerve was stimulated, and it disappeared immediately when vagal stimulation was discontinued. Stimulation of the vagus nerve with a weaker faradic current caused a shorter RP interval and therefore failed to release the return extrasystoles.

The question concerning the pathway over which the stimulus returns cannot as yet be definitely answered. The evidence of older experiments seemed to suggest that the return takes place in the upper part of the auriculoventricular node.[7] This possibility was originally considered by Drury. The sharp difference in the histologic structure between the auricular and the ventricular part of the auriculoventricular node may warrant the assumption of a difference in function. However, it seems inconceivable that the stimulus conducted from the auriculoventricular node back to the auricle would on its return to the ventricle utilize exactly and exclusively the same path. One must assume that at least the upper part of the conduction system is divided longitudinally into two paths (fig. 2). Many junctional fibers exist between the auriculoventricular node and the auricle, and, as conduction within the auriculoventricular node itself appears to be a complicated process, this functional dissociation may well occur.

The fact that, at least in part, different pathways are utilized was shown by other observations. Thus, in figure 3 *E*, an auriculoventricular rhythm existed, initiated by clamping the sinus node and its arteries. During stimulation of the left vagus nerve with a weak faradic current an inverted P wave became visible between the QRS interval and the T wave; usually it appeared 0.12 second after the beginning of the R wave. On three occasions the RP distance was longer than 0.12 second, and a return extrasystole followed. The distances between the auriculoventricular beats directly following each other at the beginning of the tracing vary only from 0.58 to 0.60 second. The distances

between the last auriculoventricular beat before and the first after the return extrasystoles in figure 3 *E* measure 0.68, 0.67 and 0.72 second. If one measures 0.60 second back from the first postextrasystolic auriculoventricular beat, a point preceding the retrograde conducted P wave is always reached. Formation of the stimulus for the auriculoventricular beat which follows the return extrasystole must therefore start at a time when the preceding auriculoventricular beat has not as yet reached the auricle, probably when this stimulus has just left the auriculoventricular node. But on its way down to the ventricle the return extrasystole itself does not disturb the auriculoventricular center. Either it is conducted downward by another path, or it does not break into the auriculoventricular center because the latter is "protectively blocked."

The shortening of the RP interval in the first auriculoventricular beat after the return extrasystole and the slight delay of the first postextrasystolic ventricular complex are consequences of the slower conduction in the bundle of His below the auriculoventricular node. At least a part of this section of the auriculoventricular system conducted three stimuli within a short period. The validity of this explanation is supported by the following facts. If the P-P distances in figure 3 *E* are measured, only small variations are found, since the auriculoventricular center is not disturbed by the return extrasystole. Slight disturbances of the interventricular intervals of the auriculoventricular beats and of the RP distances are produced by conduction disturbances in the lower parts of the auriculoventricular system.

This undisturbed action of the auriculoventricular center was, however, frequently absent. The failure of stimuli conducted from the auricle to the ventricle to disturb the action of an auriculoventricular center has been noted by Lewis and White[9] and Rothberger and Winterberg.[10]

COMMENT

These experiments show that under certain conditions a return extrasystole (or reciprocating beat) may occur in dogs and present the picture of a continuous bigeminal or trigeminal rhythm. The "method of extrasystole," which was instrumental in producing a reciprocating rhythm in Mines's experiments with the hearts of fish and of frogs, has been useful in experiments on mammalian hearts which have a small auriculoventricular connection. The method of inducing return extrasystoles in an auriculoventricular rhythm by vagal stimulation

10. Rothberger, C. J., and Winterberg, H.: Ueber Extrasystolen mit kompensatorischer Pause bei Kammerautomatie und über die Hemmungswirkung der Extrasystolen, Arch. f. d. ges. Physiol. **146**:385, 1912.

is an imitation of the clinical experience in which the same disturbances are manifested during carotid pressure or digitalization in patients with an auriculoventricular rhythm. With both methods, the RP interval of the auriculoventricular beats is prolonged, and thus the essential condition for the appearance of the return extrasystole is present. The similarity between the clinical observations and experimental tracings is striking. At present it is not possible to decide whether the prolongation of the RP interval alone is sufficient to cause the appearance of return extrasystoles. It is probable that functional longitudinal dissociation of the upper part of the auriculoventricular system is not always present but results from the extrasystoles or vagal stimulation. Once this dissociation develops, the return extrasystole can always appear provided the RP interval has a certain length; otherwise the return extrasystole would not be conducted to the ventricle by the lower part of the auriculoventricular system which is still refractory and in which no longitudinal dissociation exists.

Functional longitudinal dissociation in the small auriculoventricular system of the mammalian heart must be assumed, since the second utilization of the same pathway within a short time seems impossible in the light of present knowledge.

It is interesting to note that in experiments on the turtle's heart, using small strips of ventricular tissue, which is, of course, much less differentiated than ventricular tissue from the mammalian heart, Schmitt and Erlanger [11] found the phenomenon of reentry under certain conditions. They called it opisthodromia. A stimulus applied to the left end of the muscle strip spread to the right and returned to the left end. Reentry appeared whenever conduction in a small pathway was possible in only one direction (unidirectional block) and the adjacent fibers were able to conduct in both directions. Older experiments also supported the idea of the occurrence of multiple pathways with varying powers of conduction in the normal direction.[12] The same phenomenon was observed in the experiments described in this paper: The RP interval occasionally was longer than the PR interval of the following return extrasystole.

The alternating retrograde conduction in figures 3 *A* and *B* is also of interest. Alternation of the normal auriculoventricular conduction

11. Schmitt, F. O., and Erlanger, J.: Directional Differences in the Conduction of Impulse Through the Heart Muscle and Their Possible Relation to Extrasystolic and Fibrillary Contractions, Am. J. Physiol. **87**:326, 1921.

12. Engelmann, T. W.: Die Blätterschicht der electrischen Organe von Raja in ihren genetischen Beziehungen zur quergestreiften Muskelsubstanz, Arch. f. d. ges. Physiol. **57**:149, 1894. Erlanger, J.: Further Studies on the Physiology of Heart Block, Am. J. Physiol. **16**:160, 1906.

has been observed on rare occasions, for example, during asphyxia [13] or during a fast tachycardia.[14] Different degrees of reversed block were included among the clinical observations of Drury.[5e]

The experiments reported suggest that premature ventricular contraction may be caused by a reentry mechanism. Although this has been seen up to the present time only during an auriculoventricular rhythm, the idea cannot be dismissed that a similar disturbance in a peripheral part of the ramifications of the auriculoventricular system may cause the same mechanism when unidirectional block and delayed conduction are present. The reentry mechanism may be situated in the right or in the left ventricle and may initiate the formation of ventricular extrasystoles during sinus rhythm. Certain observations [15] seem to show, however, that, at least under some conditions, extrasystoles are evoked by an abnormal formation of stimuli independent of a disturbance of the conduction of the stimulus.

A longitudinal dissociation in the auriculoventricular conduction system with faster conduction in one bundle branch may explain tracings with the syndrome of Wolff, Parkinson and White [16] (Cooke and White, personal communication).

SUMMARY

An auriculoventricular rhythm was produced in dogs' hearts in situ; functional longitudinal dissociation appeared after the conduction system was fatigued by means of a series of extrasystoles or during stimulation of a vagus nerve. In this stage return extrasystoles (reciprocating beats) appeared whenever the RP interval of the auriculoventricular beats was prolonged. The experiments prove that under certain conditions bigeminal or trigeminal rhythm may be due to a reentry mechanism.

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13. Lewis, T., and Mathison, G. C.: Auriculo-Ventricular Heart-Block as a Result of Asphyxia, Heart **2**:47, 1910.

14. Lewis, T.; Feil, H. S., and Stroud, W. D.: Some Effects of Rhythmic Stimulation of the Auricle, Heart **7**:247 1920. Kaufmann, R.; Rothberger, C. J., and Kauf, E.: Ueber die Entstehungsarten allorhythmischer Ventrikeltätigkeit bei Vorhofflattern, Ztschr. f. d. ges. exper. Med. **51**:766, 1926.

15. Scherf, D.: Ueber den Zusammenhang zwischen festgekuppelten Extrasystolen und extrasystolischen Tachykardien, Ztschr. f. d. ges. exper. Med. **70**:375, 1930.

16. Wolff, L.; Parkinson, T., and White, P. D.: Bundle Branch Block with Short P-R Interval in Healthy Young People Prone to Paroxysmal Tachycardia, Am. Heart J. **5**:685, 1930.

RELATION OF LIVER FUNCTION TO CIRRHOSIS OF LIVER AND TO ALCOHOLISM

COMPARISON OF RESULTS OF LIVER FUNCTION TESTS WITH DEGREE OF ORGANIC CHANGE IN CIRRHOSIS OF LIVER, AND WITH RESULTS OF SUCH TESTS IN PERSONS WITH ALCOHOLISM WITHOUT CIRRHOSIS OF LIVER

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LOS ANGELES

The value of liver function tests remains in doubt. Too frequently the physician who is confronted by a difficult problem in diagnosis has liver function tests made when they are not indicated and is then left in confusion as to what interpretation to place on the results when they have been obtained. In this paper an effort is made to correlate the results of function tests with anatomic changes in the liver. In all except 1 of the cases studied, the diagnosis of cirrhosis of the liver was confirmed by examination with the peritoneoscope.[1] In all but 3 instances, a piece of the liver was removed for microscopic examination, which made it possible to compare the results of function tests with the severity of the lesion as determined by the pathologist. Classification of the changes found by the pathologist as chronic or subacute cirrhosis is purely arbitrary. The diagnosis of subacute cirrhosis implies that the disease process has not reached a terminal stage as indicated by the reactions of the tissue to the damaging agent or agents, but the determination of the changes histologically does not measure or enable one to predict the functional capacity of the liver in question. From the therapeutic viewpoint, a patient who as a result of biopsy is found to have subacute cirrhosis still has significant chances of responding to correct management.

The patients studied in this work had the following tests made on them—after biopsy of the liver had been done: determination of total serum proteins and the albumin-globulin ratio; the hippuric acid test; the bromsulphalein test, except for those patients who were jaundiced, and determination of serum cholesterol and cholesterol esters. In a few

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1. Service of Dr. John Ruddock.

instances, the Takata-Ara, galactose tolerance or urobilinogen test was made before biopsy of the liver had been done, but no great importance was attached to the results of these procedures.

METHOD

It is not out of place to consider briefly the tests used.

Serum Proteins.—These substances are important in this problem because one of the most significant alterations in metabolism associated with chronic hepatitis is the disturbance in their formation. Amino acids brought to the liver from the intestine are not converted into serum proteins in adequate amounts; consequently their concentration gradually decreases as the liver tissue is destroyed and its function fails. When the concentration of serum proteins reaches a critical level, edema, and especially ascites, follows. Addis, Poo and Lew [2] found that during two days of starvation rats lost 20 per cent of the protein from the liver and only 4 per cent of the protein from the kidneys, heart and other organs. The effect of partial hepatectomy on the blood proteins of rats was studied by Chanutin and his associates.[3] They found that on the first day following operation the blood plasma, fibrin, albumin and globulin were reduced and that by the third day the concentration of fibrin and globulin was above normal and remained so, but that the concentration of albumin was decreased and remained so during the thirty day period of observation. Decreased concentration of serum proteins in association with a diseased condition of the liver was emphasized by Salvesen.[4] He found that serum albumin decreased until it was equal to or less than the globulin fraction. He studied 16 female patients as controls and found their albumin-globulin ratio ranged between 1.26 and 2.00 with an average of 1.62; a similar study of 16 male patients gave ratios between 1.43 and 2.26, with an average of 1.72. His patients who had cirrhosis of the liver were found to have ratios between 0.30 and 0.89. Observations by Foley, Keeton, Kendrick and Darling [5] led them to state that the decrease of serum albumin in this condition is not the result of mechanical removal, but that serum proteins may pass from the blood into large accumulations of ascitic fluid without altering their concentration in the blood. In a study of patients who had nephrosis, Barker and Kirk [6] found that there was a daily loss of from 5 to 6 Gm. of protein and that a daily loss of 4 Gm. was accompanied by a reduction in concentration of serum proteins. It has been observed that the albumin-globulin ratio is reversed in patients who

2. Addis, T.; Poo, L. J., and Lew, W.: Protein Loss from the Liver During a Two-Day Fast, J. Biol. Chem. **115**:117-118 (Aug.) 1936.

3. Chanutin, A.; Hortenstine, J. C.; Cole, W. S., and Ludwig, S.: Blood Plasma Proteins in Rats Following Partial Hepatectomy and Laparotomy, J. Biol. Chem. **123**:247-256 (March) 1938.

4. Salvesen, H. A.: Variations in Plasma Protein in Non-Renal Conditions, Acta med. Scandinav. **72**:113-123, 1929.

5. Foley, E. F.; Keeton, R. W.; Kendrick, A. B., and Darling, D.: Alterations in Serum Protein as an Index of Hepatic Failure, Arch. Int. Med. **60**: 64-76 (July) 1937.

6. Barker, M. H., and Kirk, E. J.: Experimental Edema in Dogs in Relation to Edema of Renal Origin in Patients, Arch. Int. Med. **45**:319-346 (March) 1930.

have nutritional edema, and under these circumstances Liu and his associates [7] found that the addition of 28 Gm. of protein would reestablish the normal level. Thompson, McQuarrie and Bell [8] studied a 2 year old girl who had an intractable edema and who was not benefited by a protein intake of from 2.5 to 3.5 Gm. per kilogram of body weight. Her urine contained no albumin. At autopsy she was found to have primary atrophy of the liver and a normal heart and kidneys. They concluded that the injured liver was incapable of fabricating the needed serum albumin and globulin.

Hippuric Acid.—The hippuric acid test of Quick [9] is a measure of the capacity of the liver to detoxify benzoic acid. By conjugation of benzoic acid with aminoacetic acid, hippuric acid is formed. The amount of hippuric acid excreted depends on the capacity of the liver to synthesize aminoacetic acid. The normal adult is said to excrete from 3.0 to 3.5 Gm. of hippuric acid in terms of benzoic acid during a period of four hours. Snell and Plunkett [10] found a definite correlation between the amount of hippuric acid excreted and that of bromsulphalein retained in 3 patients who had portal cirrhosis. Fifty patients who present no evidence of disease of the liver or the biliary tract were studied as controls by Yardurman and Rosenthal.[11] These patients each excreted from 3 to 4 Gm. of hippuric acid. The test was found to be of value for patients who suffered from heart failure or impaired renal function, but it was found to be decisive in differentiating hepatic from extrahepatic jaundice, since the patient who has early obstructive jaundice eliminates hippuric acid normally. Lindeboom [12] found the test positive for patients who had primary disease of the liver but found also a diminished output of hippuric acid from those who had obstructive jaundice, heart failure and renal disease. In 30 controls he found the output to be from 2.97 to 4.33 Gm., with an average of 3.73 Gm. He concluded that in a study of disease of the liver the hippuric acid and galactose tolerance tests cannot be relegated to the same category and that there is no exact parallel between the result of Quick's test, the concentration of the serum proteins, and the Takata-Ara reaction.

The urine of those patients to whom this test was applied was treated by the precipitation method of Weichselbaum and Probstein.[13]

Bromsulphalein Test.—When this test was introduced by Rosenthal and White [14] they recommended the intravenous injection of 2 mg. of the dye per kilogram of

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7. Liu, S. H.; Chu, H. I.; Wang, S. H., and Chung, H. L.: Nutritional Edema, Chinese J. Physiol. **6**:73-94 (Feb. 15) 1932.

8. Thompson, W. H.; McQuarrie, I., and Bell, E. T.: Edema Associated with Hypogenesis of Serum Protein and Atrophic Changes of the Liver, J. Pediat. **9**:604-619 (Nov.) 1936.

9. Quick, A. J.: The Synthesis of Hippuric Acid: A New Test of Liver Function, Am. J. M. Sc. **185**:630-635 (May) 1933.

10. Snell, A. M., and Plunkett, J. E.: The Hippuric Acid Test for Hepatic Function, Am. J. Digest. Dis. & Nutrition **2**:716-721 (Feb.) 1936.

11. Yardurman, K., and Rosenthal, P. I.: Hippuric Acid Elimination as a Test of Liver Function, J. Lab. & Clin. Med. **22**:1046-1055 (July) 1937.

12. Lindeboom, G. A.: Die Hippursäuresynthese als Leberfunktionsprobe, Acta med. Scandinav. **99**:147-161, 1939.

13. Weichselbaum, T. E., and Probstein, J. G.: Determination of Hippuric Acid in the Urine, J. Lab. & Clin. Med. **24**:636-639 (March) 1939.

14. Rosenthal, S. M., and White, E. C.: Clinical Application of the Bromsulphalein Test for Hepatic Function, J. A. M. A. **84**:1112-1114 (April 11) 1925.

body weight. O'Leary, Greene and Rowntree[15] considered their results more accurate when they used 5 mg. per kilogram of body weight. Twenty-two of their patients who were considered to have syphilitic cirrhosis of the liver were found to have dye retention at the end of thirty and sixty minute intervals. Retention of the dye, according to the results of Robertson, Swalm and Konzelmann,[16] indicates impairment of liver function as accurately as any other test employed. Soffer[17] found results of this test were positive in 55 to 65 per cent of all patients tested who had disease of the liver. They were positive in a still higher percentage of patients who had diffuse parenchymal damage, such as results from portal cirrhosis, biliary cirrhosis and prolonged passive congestion. The findings of Magath[18] led him to the conclusion that the degree of hepatic damage is more accurately indicated by the bromsulphalein test than by the Takata-Ara or the van den Bergh test or by the albumin-globulin ratio.

The test recently has been modified by White and his co-workers.[19] They recommend the withdrawal of samples of blood at intervals of two, five and fifteen minutes after injection of 2 mg. of the dye per kilogram of body weight. Less than 5 per cent of the dye is retained normally at the end of the fifteen minute period. In the study reported here the test consisted of injection of 5 mg. of dye intravenously and withdrawal of a sample of blood thirty minutes later for determining the amount of dye retained. MacDonald[20] withdrew blood samples at five minute intervals from normal controls and determined that a dose of 10 mg. per kilogram of body weight required sixty minutes for the liver to dispose of the dye, and that with a dose of 6 mg. per kilogram thirty-five minutes was necessary. When 5 mg. was injected, all 25 controls were free of bromsulphalein at the end of twenty-five minutes. It is safe to conclude that any dye retention at the end of thirty minutes is abnormal.

Cholesterol.—Cholesterol esters are said to constitute from 50 to 70 per cent of the normal total cholesterol content of the blood plasma. Mancke[21] reported normal cholesterol values in the plasma of patients who had cirrhosis of the liver, and hypercholesteremia with complete absence of the ester fraction in those who had subacute atrophy. Those who had atrophic cirrhosis and were not cholemic were found by Epstein[22] to have but little impairment of liver function;

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15. O'Leary, P. A.; Greene, C. H., and Rowntree, L. G.: Diseases of Liver: Various Types of Syphilis of Liver with Reference to Tests for Hepatic Function, Arch. Int. Med. **44**:155-193 (Aug.) 1929.

16. Robertson, W. E.; Swalm, W. A., and Konzelmann, F. W.: Functional Capacity of the Liver, J. A. M. A. **99**:2071-2078 (Dec. 17) 1932.

17. Soffer, L. F.: Present Day Status of Liver Function Tests, Medicine **14**: 185-254 (May) 1935.

18. Magath, T. B.: Takata-Ara Test of Liver Function, Am. J. Digest. Dis. & Nutrition **2**:713-716 (Feb.) 1936.

19. White, F. W.; Daitsch, E., and Maddock, S.: The Comparative Value of Serial Hippuric Acid Excretion, Am. J. Digest. Dis. **6**:603-610 (Nov.) 1939.

20. MacDonald, D.: A Practical and Clinical Test for Liver Reserve, Surg., Gynec., & Obst. **69**:70-82 (July) 1939.

21. Mancke, R., cited by Cantarow, A: Cholesterol Metabolism, Internat. Clin. **1**:237-279 (March) 1935.

22. Epstein, E. Z.: Cholesterol of Blood Plasma in Hepatic and Biliary Diseases, Arch. Int. Med. **50**:203-222 (Aug.) 1932.

the ability to convert cholesterol into esters was within normal limits. Stroebe's [23] findings indicated that the concentration of cholesterol esters in the blood is by no means invariably reduced by severe hepatic disease, but he considered that a continuous decline of these esters is indicative of progressive damage to the liver.

In the tests reported here cholesterol was determined by Bloor's method and the cholesterol esters by the method of Weiss.

The Patients Studied.—According to the procedures mentioned on the first page, a diagnosis of cirrhosis of the liver was made in the cases of 42 patients. Thirty-two were men and 10 were women, and their ages varied from the third to

TABLE 1.—*Data Concerning Forty-Two Patients Studied*

	Number of Patients	Percentage
Sex		
Males	33	
Females	9	
Total	42	
Age in decades		
2d to 3d	1	
3d to 4th	9	
4th to 5th	9	
5th to 6th	10	
6th to 7th	9	
7th to 8th	4	
Contributory etiologic factors		
Alcoholism	28	70
Typhoid	3	7
Malaria	2	5
Syphilis	4 positive Wassermann reactions	10
Duration of illness		
Up to 4 weeks	8	
From 1 month to 6 months	15	
From 6 months to 2 years	10	
Over 2 years	3	
Total	36	
Ascites	32	
Jaundice	18	42
Classification of the cases of the forty-two patients		
Subacute	17	
Chronic	18	
Undifferentiated	4	
Hemachromatosis	1	
Syphilitic hepar lobatum	2	

the eighth decade. Of these patients, the condition in 18 was classified as chronic and in 17 as subacute. The conditions in 4 were unclassified; 2 patients had syphilis and hepar lobatum, and 1 had hemochromatosis. Practically all stages of cirrhosis were represented. Symptoms had been of short duration, 23 of 36 patients having been sick for six months or less. Alcoholism was considered to be a contributing factor in a minimum of 28 patients; the blood of 4 gave a positive Wassermann reaction, and 3 had had typhoid fever. Thirty-two had ascites; 8 had been jaundiced, and 10 were jaundiced at the time of study. Some details of these conditions are contained in table 1. Details of the results obtained in the study are recorded in table 2, and a summary of the results is found in table 3.

23. Stroebe, F.: Zur Cholesterinämie bei Lebercirrhose und hepatocellulärem Ikterus, Klin. Wchnschr. **11**:636-639 (April 9) 1932.

TABLE 2.—*Detailed Results of Examination of 42 Patients Studied*

Patient	Sex	Age	Ascites	Jaundice	Van Den Bergh Reaction and Icteric Index	Bromsulphalein Retention	Hippuric Acid	Non-protein Nitrogen	Serum Protein	Serum Albumin	Serum Globulin	Albumin/Globulin	Cholesterol and Esters	Diagnosis: Peritoneoscopic Examination	Diagnosis: Biopsy
1	M	..	0	..	13 units	....	2.5 Gm.	40	.....	...	...	....	... ..	Atrophic cirrhosis; hydrops of gallbladder; splenomegaly	Probably hyperplastic nodule in cirrhotic liver
2	M	66	2,500 cc., 12 wks.	..	.....	50%	.....	..	.....	...	...	....	... ..	Atrophic cirrhosis	Chronic cirrhosis, Laennec's type
3	M	39	3,000 cc.	..	4 units	30%	4.3 Gm.	..	.....	3.0 2.8	2.0 1.9	1.5 1.47	... ..	Cirrhosis	Hyperplastic nodule; possible cirrhosis
4	M	29	0	0	.....	Less than 5%	4.97 Gm.	..	.....	...	...	....	... ..	Fibrosis of liver capsule	Chronic cirrhosis, Laennec's type
5	F	46	2,000 cc.	+	21 units 2.3 mg. Takata-Ara ++++	1%	3.3 Gm.	..	.....	2.0	2.7	0.74	... ..	Atrophic cirrhosis	Histologic findings normal; biopsy unsatisfactory
6	M	66	10,000 cc.	0	.....	30%	3.94 Gm.	..	.....	...	...	....	... ..	Advanced cirrhosis	Chronic cirrhosis, Laennec's type
7	M	..	.....	0	5 units	100%	1.18 Gm.	28	.....	3.8	3.1	1.22	... ..	Perihepatitis associated with fibrosis of liver capsule	Chronic cirrhosis; chronic perihepatitis
8	F	66	10,000 cc.	+	73 units 6.7 mg. 111 units 17 mg. Takata-Ara +	....	.....	26	.....	2.3	3.3	0.66	192 50 (25%)	Atrophic cirrhosis, far advanced	Chronic cirrhosis with rupture of esophageal varices; bronchopneumonia; autopsy
9	M	..	++	0	7 units	30%	2.13 Gm.	..	6.4 mg.	...	...	....	192 74 (38%) 208 55 (21%)	Cirrhosis	Chronic cirrhosis, Laennec's type
10	M	39	+	0	9 units Takata-Ara +	25%	2.9 Gm.	21	.....	2.7	2.9	0.90	227 78 (34%)	Cirrhosis and splenomegaly	Chronic cirrhosis, Laennec's type

11	M	57	+	0	.....	20%	2.5 Gm.	..	7.1 mg.	...	...	....	...	..	.....	Chronic cirrhosis, Laennec's type
12	M	54	15,000 cc.	..	.....	75%	.....	..	.....	...	...	....	...	..	Advanced cirrhosis; nodular liver	Chronic cirrhosis, Laennec's type; pulmonary tuberculosis, at autopsy
13	M	65	20,000 cc., 3 mo.	0	8 units	25%	.....	..	.....	1.9	2.9	0.65	208 (30%)	63	Cirrhosis	Chronic alcoholic cirrhosis
14	F	70	6,000 cc.	+++	57 units 6.8 units	....	1.5 Gm.	..	.....	2.15	3.4	0.62	161	..	Atrophic cirrhosis; prothrombin, 75%; nodular liver, hobnailed	Chronic cirrhosis
15	M	48	50,000 cc., 19 taps	0	.....	50%	1.7 Gm.	..	6.6 mg.	...	...	....	156 Total lipids: 380	Trace	Cirrhosis; fat content of ascitic fluid, 2 Gm. in each 100 cc.	Chronic cirrhosis
16	M	58	7,000 cc., 5 mo.	0	10.5 units	70%	1.86 Gm.	..	5.3 mg.	2.5	0.3	0.83	208 161	..	Atrophic cirrhosis	Laennec's cirrhosis, far advanced
17	M	48	8,000 cc., 5 mo., 8 taps	..	.....	40%	2.2 Gm.	27	7.0 mg.	2.0	2.3	0.87	156	..	Chronic cirrhosis; hobnailed liver	No biopsy
18	M	38	4,000 cc.	..	.....	....	4.76 Gm.	..	5.7 mg.	2.4 2.2	1.9 3.1	1.26 0.70	131 (87%) Lipids: 542	113	Cirrhosis; galactose tolerance, 1.3 Gm.	Hemachromatosis
19	M	..	3,000 cc.	..	12.5 units	55%	2.8 Gm.	..	6.5 mg.	2.6	4.2	0.61	161	37	Subacute cirrhosis; galactose tolerance, 0.6 Gm.; dextrose tolerance, 1 hr. 78, 2 hr. 109, 3 hr. 91, 4 hr. 101	Late subacute cirrhosis; no autopsy at death of patient
20	F	56	0	++ 7 mo. ago	9.5 units 0.9 mg.	50%	.....	..	.....	...	...	....	...	..	Subacute hepatitis; early cirrhosis	Subacute hepatitis
21	M	70	.....	..	.....	50%	.....	..	.....	...	...	....	...	..	Cirrhosis	Late subacute alcoholic cirrhosis
22	M	39	.....	0	7 units	....	3.64 Gm.	..	.....	3.5	3.1	1.12	...	..	Subacute cirrhosis	Subacute alcoholic cirrhosis
23	M	62	4,000 cc., 5 mo.	++	16 units	60%	2.64 Gm.	..	.....	2.2	3.8	0.58	132	..	Cirrhosis with splenomegaly	Late subacute alcoholic cirrhosis
															Anatomic diagnosis: weight of liver, 1,060 Gm.; weight of spleen, 870 Gm.; portal cirrhosis; ascites, benign; nephrosclerosis; rheumatic mitral stenosis	
24	M	67	1,300 cc.	0	6 units	Less than 15%	4.64 Gm.	29	.....	2.8	2.6	1.07	192 (60%)	117	Subacute chronic cirrhosis	Laennec's cirrhosis

TABLE 2.—*Detailed Results of Examination of 42 Patients Studied—Continued*

Patient	Sex	Age	Ascites	Jaundice	Van Den Bergh Reaction and Icteric Index	Bromsulphalein Retention	Hippuric Acid	Nonprotein Nitrogen	Serum Protein	Serum Albumin	Serum Globulin	Albumin/Globulin	Cholesterol and Esters	Diagnosis: Peritoneoscopic Examination	Diagnosis: Biopsy
25	M	33	Small amount of fluid	0	.....	....	.....	..	.....	...	...	....	200 117 (59%) Lipids: 968	Subacute Laennec's cirrhosis; dextrose tolerance 92 at 7:15, 104 at 8:15, 81 at 9:15 and 85 at 10:15; galactose, trace; liver enlarged	Subacute alcoholic cirrhosis (fatty)
26	M	36	20,000 cc.	0	13.3 units 10.8 units	50%	1.12 Gm.	..	6.6 mg.	2.2	3.8	0.57	204 ..	Subacute cirrhosis; extremely enlarged liver	Subacute alcoholic cirrhosis
27	M	55	.....	++	57 units 5.3 mg.	Less than 15%	4.1 Gm.	..	.....	...	...	....	370 200 (54%)	Subacute cirrhosis; large granular liver	Subacute alcoholic cirrhosis; operation for obstruction of the common duct at ampulla; probable neoplasm, benign or malignant
					19 units 1.5 mg.	....	2.79 Gm.								
					12 units 0.07 mg.										
28	M	41	+++	..	.....	....	3.55 Gm.	..	.....	...	...	....	... ..	Late subacute alcoholic cirrhosis	Late subacute alcoholic cirrhosis
29	M	42	1,300 cc.	0	2.9 mg. 38 units	....	1.5 Gm.	..	.....	3.2	2.9	1.09	125 48 (39%)	Subacute alcoholic cirrhosis; galactose tolerance, 0.04 Gm.; phosphatase, 6.8 units; phosphorus, 2.4	Subacute alcoholic cirrhosis
					9 units	....	.....	..	.....	...	...	....	178 78 (43%) B. lip.: 518		
30	M	..	+++	++	7.2 mg. 67 units	....	4.60 Gm.	..	.....	2.9	2.9	1.06	163 Slight trace Lipids: 716 mg.	Subacute cirrhosis	Subacute alcoholic cirrhosis; fibrosis +++; fat +++; necrosis of liver cells ++; galactose tolerance, 0.4 Gm.
					6.3 mg. 62 units								219 135 (62%) Lipids: 978		
					2.2 mg. 26 units								238 107 (45%) Lipids: 800		
					22 mg.										
					1.7 mg. 15.7 units										
31	M	33	3,500 cc.	++	.....	....	.....	..	.....	3.5	4.2	0.83	278 ..	Subacute cirrhosis with ascites	Subacute alcoholic cirrhosis

32	M	50	4,000 cc., 12 yr.	0	8 units	75%	.....	..	.....	2.5	2.9	0.86	...	..	Cirrhosis	Subacute alcoholic cirrhosis; death from ascites, esophageal varices with hemorrhage, and intestinal obstruction
33	F	49	7,000 cc.	0	33 units 2.2 mg. 28.5 units Takata-Ara + 5.5 mg. 51 units 20 units	....	.....	..	.....	1.4 1.6 1.5 1.8 1.4	3.8 3.8 3.8 3.3 2.9	0.36 0.42 0.59 0.54 0.48	147 128 166	.. Trace	Subacute cirrhosis; blood sugar, 299 mg. in each 100 cc.; amino acid nitrogen, 6.2 mg.	Late subacute cirrhosis; hemachromatosis
34	M	47	5,500 cc.	0	5.7 units	37%	1.55 Gm.	..	5.8 mg.	...	...	....	200 (24%)	47	Subacute cirrhosis; liver moderately enlarged	Late subacute cirrhosis
35	M	46	0	++	53.2 units 40 units 13 units	....	2 Gm.	..	8.6 mg.	...	...	....	178	..	Acute hepatitis; liver slightly enlarged; galactose, trace	Laennec's cirrhosis; subacute hepatitis; left hospital well
36	M	73	0	++	40 units	....	1.36 Gm.	..	.....	2.6	2.9	0.9	208	..	Subacute cirrhosis; granular liver	Subacute cirrhosis; autopsy
37	F	46	1,900 cc., 9 mo.	0	.....	0%	2.96 Gm.	30	6.3 mg.	3.1 4.0 2.9 2.7	3.1 3.0 1.3 2.9	1.0 1.33 2.2 0.93	... 178	..	Cirrhotic changes on the basis of long-standing chronic passive congestion	Chronic cirrhosis, type unknown; final impression, coronary disease with posterior infarction; autopsy
38	F	52	.....	..	7 units	20%	4 Gm.	..	.....	2.6	2.3	1.13	500	..	Cirrhosis	No biopsy
39	M	57	4,500 cc.	0	5 units	50%	2.3 Gm.	..	.....	...	...	....	...	..	Nodular cirrhosis; hepar lobatum	Gumma of liver
40	F	69	10,000 cc.	+	5.5 units	55%	1.33 Gm.	..	5.7 mg.	3.1	2.6	1.19	208	90	Cirrhosis, probably syphilitic	Cirrhosis, syphilitic; hepar lobatum; death from condition of liver; autopsy
41	F	67	.....	..	4.4 units	Less than 10%	0.9 Gm.	..	.....	2.4	4.2	0.57	172	..	No peritoneoscopic examination	Hypertensive heart disease; blood pressure 225/130; duodenal ulcer; gastric hemorrhage; roentgen studies
42	M	56	6,000 cc.	..	5 units	40%	0.88 Gm.	..	10.5 mg.	.	...	....	114	..	Atrophic cirrhosis; hobnail liver	Late subacute or chronic cirrhosis; hypertensive heart disease and chronic cirrhosis with ascites at autopsy

RESULTS

A summary of the results of liver function tests done is found in table 3. From a study of this and of the details in table 2, it is seen that the most reliable results are those obtained by the bromsulphalein test and by determination of the serum proteins and the albumin-globulin ratio.

If one makes the liberal allowance of 10 per cent retention of bromsulphalein thirty minutes after the intravenous injection of 5 mg. per kilogram of body weight as being within normal limit, it is seen that in 22, or 78 per cent, of the 28 patients tested the results indicate definitely impaired liver function. None of these patients was jaundiced when the test was made.

TABLE 3.—*Results of Tests*

Number of Patients	Test	Result
28	Bromsulphalein	Positive in 22 patients Negative in 6 patients
32	Hippuric acid *	Positive in 18 patients Negative in 14 patients
21	Albumin-globulin	Positive in 15 patients Negative in 6 patients
16	Cholesterol and esters	Positive in 9 patients Negative in 7 patients
6	Takata-Ara	Positive in 4 patients Negative in 2 patients
10	Jaundice at time of interview	

* The result of the hippuric acid test was negative in 4 patients who gave positive reactions to the bromsulphalein tests and positive in 1 instance in which a negative reaction to the bromsulphalein test was obtained.

The total serum proteins and the albumin-globulin ratio were determined in 21 patients. In 15 of these, 72 per cent, the total proteins were definitely below normal and the ratio of albumin to globulin was reversed.

The hippuric acid test was done on 32 patients, and in 18, or 56 per cent, of these the results indicated impaired liver function. Blood cholesterol and cholesterol esters were determined in 16 patients, and in 9, or 56 per cent, of these the results indicated impaired liver function. It should be mentioned that the methods used in the last determinations are tedious and subject to considerable error.

From the standpoint of prognosis, the importance of failure to excrete bromsulphalein, reduction in serum proteins and reversal of the albumin-globulin ratio in the same patient should be emphasized. None of the patients in this group who were found to have these conditions lived longer than six months. It may be said that these findings indicate a high degree of damage to the endothelial system of the liver and to the ability of the liver to synthesize proteins and that under these circumstances, nothing more than temporary improvement of the patient's health can be expected.

COMMENT

Causes of Impaired Liver Function.—It becomes increasingly apparent that liver function bears a direct relation to the state of nutrition of the organ. A deficiency of glycogen renders the liver especially susceptible to injury. According to Moon [24] the following agents are capable of producing cirrhosis of the liver: bacterial infections (toxins?), manganese chloride plus phenylhydrazine, carbon tetrachloride, tars and phosphorus plus alcohol. An abnormal degree of fatty change is found in the livers of malnourished, emaciated persons. Increased amounts of fat in the hepatic cells of starving animals were described by Dible and Libman.[25] In rats whose fat reserve was meager the increased fat in the liver was only relative, but when the fat stores were sufficient to permit mobilization, an absolute increase was found in the liver. The condition is found in the livers of those who suffer from diseases of the intestinal tract that produce diarrhea. Best and Campbell [26] stated that fatty livers may be produced in experimental animals by fasting, by feeding diets rich in fats, by feeding cholesterol and by phosphorus poisoning. Injection of pituitary substance has been found to be followed by increased amounts of fat in the livers of fasting and fed rats. Friedenwald [27] found that after prolonged administration of alcohol to rabbits their livers contained abnormal amounts of fat. This change was reversible, and the fat would disappear soon after the administration of alcohol was stopped. The presence of fat in the liver, with few exceptions, was considered abnormal by Connor.[28] He stated that under certain circumstances fat may accumulate in the human liver within forty-eight hours and disappear rapidly after the administration of dextrose. It is also maintained that at autopsy as much as 60 per cent of the livers of chronic alcoholic addicts may consist of fat, and that if this condition persists for a sufficient length of time the sinusoids collapse, portal circulation becomes blocked and parenchymatous anoxemia follows. It has been demonstrated by Bollman [29] that fatty livers develop in fasting dogs and other experimental animals living

24. Moon, V. H.: Experimental Cirrhosis in Relation to Human Cirrhosis, Arch. Path. **18**:381-424 (Sept.) 1934.

25. Dible, J. H., and Libman, J.: Further Observations on Fat Mobilization in Starvation, J. Path. & Bact. **38**:269-284 (May) 1934.

26. Best, C. H., and Campbell, J.: Anterior Pituitary Extracts and Liver Fat, J. Physiol. **86**:190-203 (Feb. 8) 1936.

27. Friedenwald, J.: The Pathologic Effects of Alcohol on Rabbits, J. A. M. A. **45**:780-784 (Sept. 9) 1905.

28. Connor, C. L.: Fatty Infiltration of the Liver and Development of Cirrhosis in Diabetes and Chronic Alcoholism, Am. J. Path. **14**:347-364 (May) 1938.

29. Bollman, J. L.: Some Experimental Observations Pertinent to Treatment of Hepatic Disease, Ann. Int. Med. **12**:2-5 (July) 1938.

on a diet high in fat and that administration of alcohol intensifies this change. He found also that if dogs were given a well balanced diet and large amounts of alcohol the liver remained normal.

The dietary deficiency usually associated with alcoholism is in itself detrimental to liver metabolism. Lipschitz [30] and his co-workers found that vitamin B_1 deficiency was followed by serious impairment of the pyruvic acid metabolism in the liver and by deranged carbohydrate metabolism in the entire organism. By a study of the food habits of 131 alcoholic addicts, Romano [31] found that 58 per cent of them had some degree of neuritis and that 79 per cent took inadequate amounts of food.

Cirrhosis of the liver may be found as frequently in some countries where alcohol is used but little as in others where its use is habitual, but a common predisposing factor is present in each instance, namely, failure of alimentary absorption. Yenikonshian [32] stated the belief that malaria and dysentery, or a combination of these, are important factors in the production of cirrhosis of nonalcoholic origin among the natives of Syria and Lebanon. Infestation by intestinal parasites is prevalent among these peoples, and the average age of those who acquire cirrhosis of the liver is much lower than it is in this country. According to a report by Rolleston and McNee [33] cirrhosis of the liver is much more frequent among the natives of India than it is among the white people. Records of 535 autopsies done on natives of Vizagapatam revéaled that 9.3 per cent had cirrhosis of the liver. Twenty-five patients were found to have portal cirrhosis. Tirumurti [34] and his co-worker emphasized the fact that the people of this locale do not drink alcohol. It was affirmed by Bloomfield [35] that cirrhosis of the liver may progress to a far advanced stage before it causes symptoms or produces changes which induce the patient to seek medical care and by the same token may cause the clinician to fail to make a correct diagnosis. In most instances a review of the history gives no indication as to the time of onset of the

30. Lipschitz, M. A.; Potter, V. R., and Elvehjem, C. A.: Metabolism of Pyruvic Acid in Vitamin B_1 Deficiency and Inanition, J. Biol. Chem. **123**:267-281 (March) 1938.

31. Romano, J.: Deficiency Syndromes Associated with Chronic Alcoholism, Am. J. M. Sc. **194**:645-651 (Nov.) 1937.

32. Yenikonshian, H. A.: Non-Alcoholic Cirrhosis of the Liver in Lebanon and Syria, J. A. M. A. **103**:660-661 (Sept. 1) 1934.

33. Rolleston, H. D., and McNee, J. W.: Diseases of Liver, Gall Bladder, and Bile Ducts, ed. 3, New York, The Macmillan Company, 1929.

34. Tirumurti, T. S., and Radhakrishna Rao, M. V.: Incidence of Portal Cirrhosis of the Liver in Vizagapatam, Indian M. Gaz. **69**:74-76 (Feb.) 1934.

35. Bloomfield, A. L.: The Natural History of Chronic Hepatitis, Am. J. M. Sc. **195**:429-444 (April) 1938.

disease. Also the prognosis is uncertain, because of a lack of knowledge concerning the complex alterations of metabolism that result from disease of the liver.

Causes of Death of Patients Who Have Cirrhosis of the Liver.—In an analysis of the clinical and autopsy records of 217 patients in the Los Angeles County Hospital who were found to have alcoholic cirrhosis of the liver, Evans and Gray [36] found the causes of death to be as follows:

Causes of Death

	No.	Per Cent
Pneumonia		
Lobular	73	37.0
Lobar	8	
Gastrointestinal hemorrhage	43	20.0
Insufficiency of the liver	26	12.0
Peritonitis	10	4.6
Miscellaneous	57	21.0

The present study includes an analysis of the autopsy records of 102 patients whose conditions were similar to those found in the aforementioned group, but which were classified as being associated with subacute or chronic cirrhosis. The causes of death in this group were tabulated as follows:

Causes of Death

	Subacute		Chronic	
	No.	Percentage	No.	Percentage
Pneumonia	16	33	15	28
Gastrointestinal hemorrhage	13	27	17	31
Insufficiency of the liver	10	20	12	22
Miscellaneous	9	20	10	19

From these figures it appears that the prognosis in patients who have cirrhosis of the liver does not correspond to the histologic picture described by the pathologist. Regardless of the probable criticism that the division of these pathologic pictures into two groups is only an approximation, the fact remains that these patients die of identical causes, and in about the same ratios, whether they have large, smooth or small, fibrotic, hobnailed livers. The classification was made according to certain arbitrary rules. If the liver weighed more than 2,000 Gm., contained an extensive amount of fat or showed evidence of necrosis, the cirrhosis was classified as subacute. If the liver was smaller, had irregular, scarred surfaces and presented unusual resistance to cutting, the cirrhosis was designated as chronic.

Hall and Morgan [37] described the subacute cirrhotic liver as being large, as weighing from 2,000 to 5,000 Gm., as having a smooth or finely

36. Evans, N., and Gray, P. A.: Laennec's Cirrhosis: Report of Two Hundred and Seventeen Cases, J. A. M. A. **110**:1159-1161 (April 9) 1938.

37. Hall, E. M., and Morgan, W. A.: Progressive Alcoholic Cirrhosis, Arch. Path. **27**:672-690 (April) 1939.

granular surface and as being usually fatty. Fibrosis is not as extensive and the tissue is more cellular than that of the liver which presents chronic atrophic cirrhosis. Necrosis of the hepatic cells is prevalent; this is conspicuously absent in the chronic form.

When the ages of these groups of patients are studied in graphs a conspicuous difference between the two conditions is seen; the peaks

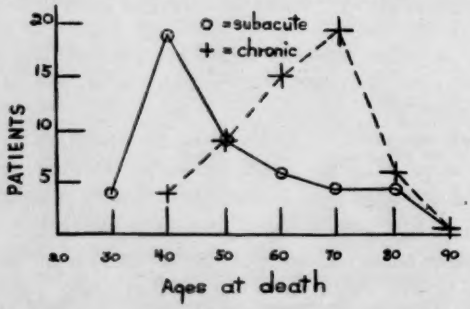

Fig. 1.—Comparison of the ages at death of patients with subacute cirrhosis of the liver and those of patients with chronic cirrhosis of the liver.

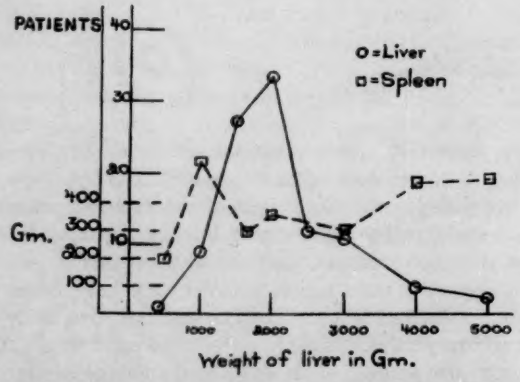

Fig. 2.—Comparison of the weight of the liver and that of the spleen in patients with cirrhosis of the liver.

indicating the age of greatest incidence of the two conditions are two decades apart (fig. 1). This is considered to be related to the pathogenesis of the separate conditions. There appeared to be no definite relationship between the weights of the liver and of the spleen (fig. 2). In chronic cirrhosis of the liver, parenchyma is replaced by fibrous tissue, and even though there is extensive regeneration and hypertrophy

of the cells of the liver, the size of the organ decreases as a result of contraction of fibrous tissue. It should be emphasized again that the liver function cannot be judged by the degree of these changes, as indicated by the gross or even the microscopic appearance of the tissue.

Studies of Liver Function in Patients with Acute Alcoholism.—After a study of liver function as indicated by a variety of tests in patients in whom biopsy of the liver proved varying degrees of cirrhosis of liver, it was decided to apply some of these same tests to a group of patients who were recovering from severe alcoholic intoxication. The 17 men selected for this study were tested within twenty-four hours after they had been detained because of intoxication. Only those who had been

TABLE 4.—*Results of Liver Function Tests in Patients with Acute Alcoholism*

Patient	Age	Retention of Bromsulphalein	Serum Protein	Serum Albumin	Serum Globulin	Cholesterol	
						Total	Esters
1	53	0	...	...	...	...	...
2	53	0	...	...	...	...	...
3	50	20%	...	...	...	...	...
4	42	5%	...	...	...	...	...
5	58	Less than 10%	6.7	3.5	2.5	...	...
6	64	Less than 10%	...	5.3	2.6	...	...
7	52	0	5.9	3.8	1.9	...	...
8	52	20%	6.4	3.5	2.0	...	...
9	31	0	6.2	4.1	2.6	...	...
10	56	Less than 10%	6.5	3.9	2.3	...	...
11	52	0	...	2.9	2.4	208	141 (67%)
12	62	10%	...	3.2	2.3	178	91 (51%)
13	40	0	...	3.9	2.6	166	61 (37%)
14	62	0	...	3.8	1.8	208	111 (47%)
15	51	20%	...	2.6	2.4	172	88 (51%)
16	49	0	...	3.8	2.8	227	111 (51%)
17	45	0	...	3.5	2.6	156	72 (46%)

drinking for two or more weeks and who had taken but small amounts of food were studied. Each patient complained of nausea and anorexia and had a pronounced tremor, but none had an enlarged liver, jaundice or evidence of peripheral neuritis.

The bromsulphalein test was done on each of the 17. Serum albumin and globulin were determined in 13, cholesterol and cholesterol esters in 7. Four of the 17 patients retained 10 per cent or more of the bromsulphalein for more than thirty minutes, which retention indicated impaired liver function. Serum albumin and globulin were within normal limits, and with one exception cholesterol esters were normal. Patient 13 did not retain an abnormal amount of dye, but his cholesterol esters were 37 per cent; 40 per cent was considered the lower limit of normal. There was no opportunity to do follow-up studies on these patients, so it can only be said that in the stage in which they were studied their ability to excrete bromsulphalein was impaired.

The results of the tests are seen in table 4.

An effort is being made to do liver function tests on those patients in whom the tests indicated a damaged condition of the liver when they are between their drinking bouts and to continue studies on them as long as possible.

SUMMARY

The liver function tests used in a study of 42 patients who had proved cirrhosis of the liver and 17 who suffered from acute alcoholism are discussed.

Some of the causes of impaired liver function are discussed briefly.

The diagnosis made as a result of the appearance of the liver as seen through the peritoneoscope and that made by the pathologist who examined the specimen taken for biopsy paralleled each other with surprising accuracy.

The established variations in degree of organic change in the livers of these patients in conjunction with the results of liver function tests indicate that the use of these tests is justified from the standpoint of diagnosis, plan of management and prognosis.

Results of the function tests used, considered with the histologic diagnosis made on the respective patients, indicate that these tests may be arranged in the following order, according to their value in clinical studies: bromsulphalein test, determination of the total serum proteins and the serum albumin-globulin ratio, hippuric acid test and determination of the serum cholesterol and the cholesterol esters.

The results of tests on patients with acute alcoholism indicate that early and perhaps temporary impairment of liver function may be detected by the bromsulphalein test. Additional studies on this type of patient are under way.

1930 Wilshire Boulevard.

FATAL BRONCHIAL ASTHMA

REPORT OF SEVEN CASES

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Death rarely comes to patients in status asthmaticus. Thieme and Sheldon,[1] summarizing the literature prior to 1937, counted only 43 cases with autopsy reports in which the patient died in an asthmatic paroxysm. They added 7 such cases and since then additional cases have been reported by Fowler [2] and by Lamson and Butt.[3]

Of 56,000 admissions to the medical service of the Peter Bent Brigham Hospital, 1,378 represented those of 992 patients who had had either a primary or a secondary clinical diagnosis of bronchial asthma; of this number 49 died. Autopsies were performed on 23 of these 49 patients. In 7 of these 23 patients the clinical picture was that of status asthmaticus, the autopsy observations were consistent with bronchial asthma and no extrapulmonary cause of death was present.

The 7 cases are herewith reported.

CLINICAL FINDINGS

The patients were all women between the ages of 24 and 67, with a mean age of 45. In 6 cases the primary clinical diagnosis was bronchial asthma. The duration of the disease was six months to fourteen years, and its onset was in most instances subsequent to an infection of the respiratory tract. There was no uniformity in regard to the time of year in which the fatal attack occurred, and its duration was also variable, ranging from two days to several weeks. The cause of the asthma in these cases was obscure and the results of cutaneous tests in 6 instances were inconclusive.

Examination in every case revealed great respiratory distress with exaggerated difficulty in expiration. Appreciable cyanosis was observed in 4 cases. In only 4 cases of the group was the anteroposterior diameter of the chest increased. In 5 cases the heart was entirely normal as far

From the Department of Pathology, the Peter Bent Brigham Hospital, Boston.

1. Thieme, E. T., and Sheldon, J. M.: Correlation of Clinical and Pathologic Findings in Bronchial Asthma, J. Allergy **9**:246-269 (March) 1938.

2. Fowler, K.: Necropsy Studies on Two Patients Dying in Asthma, Pennsylvania M. J. **40**:720-724 (June) 1937.

3. Lamson, R. W., and Butt, E. M.: Fatal "Asthma": Clinical and Pathologic Consideration of One Hundred and Eighty-Seven Cases, J. A. M. A. **108**:1843-1850 (May 29) 1937.

TABLE 1.—*Results of Clinical Study of Seven Patients Who Died of Asthma*

Name	A. D.	L. McD.	B. B.	I. B.	R. M.	E. K.	G. C.
Age	45	53	40	45	49	23	67
Occupation	None	Housewife	Housewife	Secretary	Housewife	None	None
Sex	Female	Female	Female	Female	Female	Female	Female
Complaint	Asthma	Dyspnea; coma	Asthma	Asthma	Asthma	Dyspnea; asthma	Asthma
Allergy, family history	None	None	None	None	None	None	Family history; hives
Allergy, past history	None	None	None	None	None	None	None
Duration of asthma	6 months	14 years	2 years	7 years	9 years	7 years	7 years
Condition preceding onset	"Bronchitis"	"Cold"	"Exposure"	"Influenza"	Pneumonia	"Cold"	.....
Seasonal or other incidence	None	Bedtime	Catamenial	None	Nocturnal	None	Fall
Cutaneous tests	None	Negative	Positive to wheat, egg and beef	Negative	Positive to green peas, pollen	Negative	Negative
Duration of terminal illness	? weeks	6 weeks	1 month	2 weeks	2 months	3 weeks	3 weeks
Date of death	Feb. 12	Feb. 21	June 9	Oct. 21	Sept. 10	July 25	Oct. 23
Findings on physical examination:							
Cyanosis	None	Appreciable	Appreciable	Appreciable	.....	None	Appreciable
Shape of chest	Barrel	Barrel	Normal	Barrel	Asthenic; pigeon breast	Long, narrow	Slightly thickened
Heart	Enlarged to left	Normal	Normal	Normal	Normal	Normal	Normal
Lungs: Percussion note	Resonant; apex dull	Hyperresonant	Resonant	Hyperresonant	Hyperresonant	Hyperresonant	Hyperresonant
Rales	Musical	Occasional dry rale	Squeaking; musical	Squeaking; groaning	Expiratory; musical	Wheezing; groaning; musical	Sibilant; sonorous
Signs simulating failure of right side of heart	Edema	.....	Edema	.....	.....	Distended veins of neck	Palpable liver?; edema; distended veins of neck
Temperature, degrees F	96 to 102	98 to 101	98.4 to 99	99 to 99.6	98 to 99	98 to 106	100.2
Pulse rate	100 to 148	92 to 130	96 to 100	105 to 135	80 to 136	88 to 140	70 to 140*
Respiratory rate	10 to 35	20 to 30	24 to 32	25 to 32	15 to 30	27 to 48	24
Blood pressure	115/80	138/88	132/92	Expiratory 160/100; Inspiratory 145/90	126/94	130/90	130/60
Electrocardiogram	Normal curves	.....	.....	.....	Normal curves	Abnormal form of ventricular complex	.....
Laboratory data:							
Red blood cells	4,000,000	5,100,000	4,100,000	5,200,000	5,300,000	5,500,000	.....
Hemoglobin (per cent)	70	80	70	96	100	100	.....
White blood cells	11,200	8,900	10,000; 3,800	15,000	7,300	17,400	.....
Polymorphonuclear leukocytes (per cent)	48	70	72	75	72	88	.....
Eosinophils (per cent)	11	1	4	0	3	0	.....
Outstanding terminal signs	Dyspnea; weakness	Cyanosis; dyspnea	Cyanosis; respiratory failure	Dyspnea	Dyspnea	Dyspnea; exhaustion; hyperpyrexia	Slow respiration; exhaustion
Respiratory failure preceding cardiac failure	.....	Yes	Yes	No	.....	.....	.....

* Cardiac rate shifted between 72 and 140 at fifteen second intervals.

as physical examination was concerned; in the other 2 cases the findings were enlargement, in 1, and a cardiac rate shifting between 72 and 140, in the other. The pulse rate was nearly always elevated, rarely ranging as low as 88 and usually lying between 100 and 140. In some instances there were signs simulating right-sided heart failure, such as a questionably enlarged liver, in 1 case, distention of the veins of the neck, in 2 cases, and dependent edema, in 3 cases.

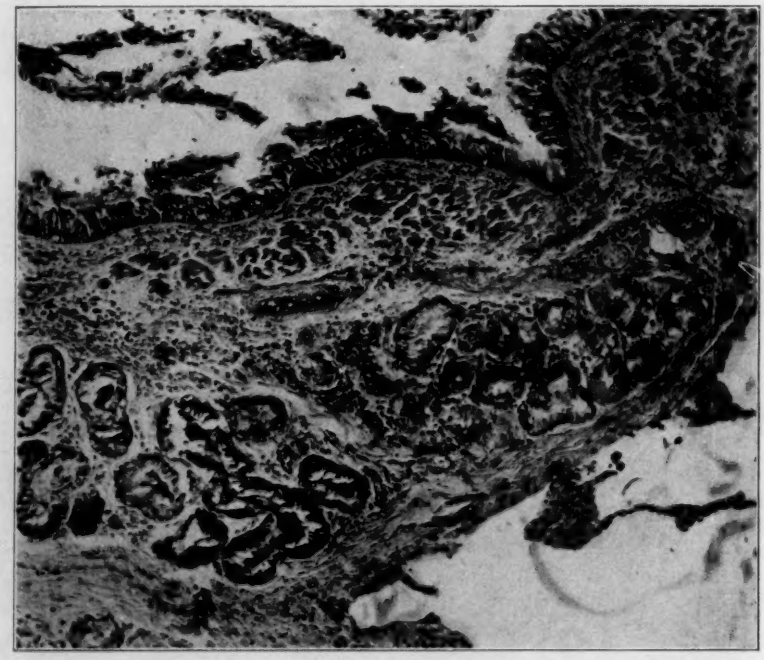

Fig. 1.—The wall of a bronchus (× 100) in a patient who died of bronchial asthma. Note the hypertrophied musculature, the bronchial glands distended with thick mucus, the hyaline basement membrane and the thickening of the submucous layer. Many of the cells are eosinophils.

The lungs were hyperresonant with sibilant and sonorous rales, wheezes and groans, but no crackling rales. The expiratory phase was prolonged, and in 1 case it was noted that the breath sounds were uniformly diminished. There was, however, no uniformity in the respiratory rate. The blood pressure was normal in all instances, and fever was observed in 4 cases. The blood showed a high eosinophil count in only 2 cases, 11 and 4 per cent, respectively. In the other 4 in which blood counts were recorded the eosinophils averaged 2 per cent.

* Cardiac rate shifted between 72 and 140 at fifteen second intervals.

In the terminal stages the patients failed to get adequate relief from epinephrine, became more dyspneic, and sometimes more cyanotic, and died. In 2 instances it was noted that the heart continued to beat for some time after respirations ceased.

The pertinent clinical findings are summarized in table 1.

POSTMORTEM OBSERVATIONS

At autopsy in every case the voluminous, feathery lungs completely filled the pleural cavity and in several instances actually bulged out over

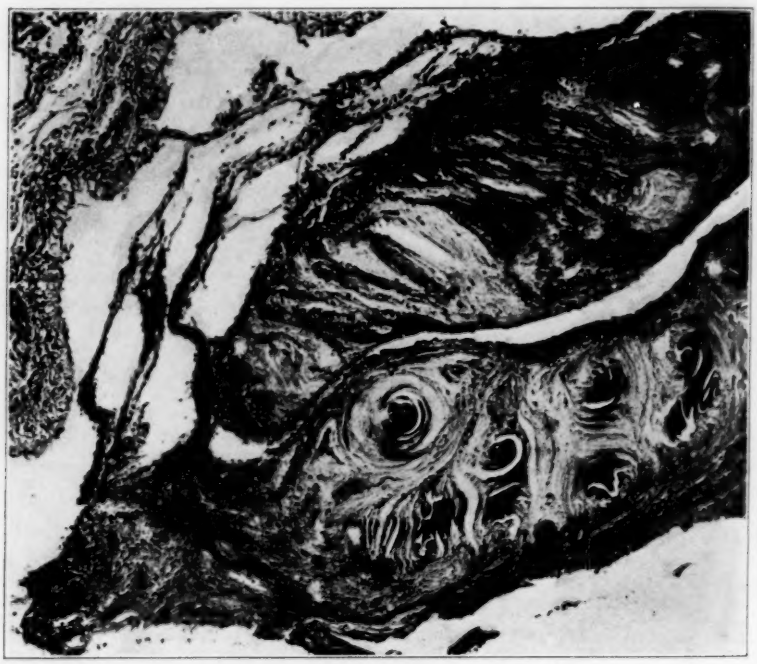

Fig. 2.—Curschmann's spiral within the lumen of a bronchus (× 50) in a patient who died of bronchial asthma.

the mediastinum on removal of the breast plate. The weight of the lungs averaged 300 Gm. each in the 4 instances in which it was determined. The cut surface showed, in all instances but 1, a large amount of thick, viscid, tenacious, elastic, mucoid material in the middle-sized (3 to 6 mm.) bronchi.

Microscopic sections showed emphysema in all cases. The severest lesions were observed in the middle-sized bronchi (fig. 1). In 6 cases

thick plugs were found in these bronchi. The plugs all contained eosinophils, and in 4 cases Curschmann's spirals were recognized (fig. 2). In 4 cases the mucus plugs in the bronchial lumen contained Charcot-Leyden crystals (fig. 3). The bronchial mucosa showed metaplasia in only 1 instance, but in 6 instances it was thrown up into folds, and frequently small sacculations of the mucosa could be seen. The mucosa and submucosa were infiltrated with lymphocytes and varying numbers of eosinophils, but the mucous glands, cartilage and musculature usually

Fig. 3.—Charcot-Leyden crystals in the mucus within the bronchial lumen (× 1,500) in a patient who died of bronchial asthma. Several appear in cross section, showing six sides. Others appear in profile, giving the appearance of a double pyramid.

escaped infiltration with such cells. In all cases the submucosal layer was widened and the basement membrane was thick and hyaline. Hypertrophy of the bronchial musculature, present in every case, varied from bronchus to bronchus, being extreme in some bronchi and absent in others. Inspissated mucus distending the bronchial glands, with pressure atrophy of the acinic cells, was frequently seen; it occurred in the 5 cases in which glands were present for examination. The amount of such

TABLE 2.—*Postmortem Observations*

Name	A. D.	L. McD.	B. B.	I. B.	R. M.	E. K.	G. C.
Macroscopic Examination:							
Thorax after opening	Lungs bulging from beds	Lungs voluminous, bulging, obscuring upper part of mediastinum	Lungs meeting in midline, obscuring mediastinum	Lungs meeting anterior to pericardium	Lungs completely filling pleural spaces	Lungs bulging	Lungs bulging over mediastinum
Pleural cavities	Apical adhesions	Apical adhesions	Apical and basal adhesions	Apical adhesions	Normal	Adhesions throughout	Normal
Lungs							
Weight (Gm.)	.....	240; 220	280; 240	.....	.....	340; 320	420; 290
Gross appearance	Grayish yellow; rounded; emphysematous	Pallid; voluminous	Voluminous	Pallid; emphysematous	Pale; distended	Doughy; retaining shape	Voluminous; doughy; one area of consolidation, several areas of atelectasis
Bronchi	Tenacious, mucinous material	Minute amount of mucoid exudate	Almost all plugged with tenacious, glairy mucus	Almost all plugged with gelatinous, tenacious mucus	Completely plugged with tenacious, thick, cloudy, adherent mucus	Mucus plugs; frothy, purulent mucus	Plugs of thick, white, elastic, tenacious, mucus
Apexes	Normal	Scarred	Scarred	Indurated	Normal	Normal	Scarred
Heart							
Weight (Gm.)	365	320	200	310	300	200	260
Chambers	Slight dilatation of right auricle	Normal	Normal	Slight prominence of right auricle and right ventricle	Normal	Normal	Normal
Thickness of musculature, left and right ventricles (mm.)	14; 5	14; 4	13; 3	15; 3	8; 4	9; 3	14; 5
Coronary arteries	Normal	Slight sclerosis	Normal	Normal	Normal	Normal	Normal
Valves	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Liver	Slight congestion ‡	Congestion	Congestion	Normal	Not examined	Congestion	Congestion

Ankles	Normal	Normal	Edema	Normal	Normal	Normal	Edema
Skin	.....	.....	.....	.....	.....	Cyanosis	Cyanosis
Other organs	No significant lesions	Chronic pancreatitis	Hyperplasia of thyroid; mild ptosis of kidney	No significant lesions	Examination limited to chest	No significant lesions	No significant lesions
Microscopic Examination:							
Lungs							
Bronchi							
Lumen	Mucus plugs; C. L.* crystals; C.† spirals; eosinophils	Almost free of mucus; few eosinophils	Mucus plugs; C. spirals; eosinophils	Mucus plugs; C. L. crystals; C. spirals; eosinophils	Mucus plugs; C. L. crystals; eosinophils	Mucus plugs; eosinophils	Mucus plugs; C. L. crystals; C. spirals; eosinophils
Mucosa	Thickened; infolded; sacculated; no metaplasia	Thickened; infolded; sacculated; no metaplasia	Thickened; infolded; sacculated; no metaplasia	No thickening, infolding, or metaplasia; sacculated	Slightly thickened; infolded; sacculated; no metaplasia	Thickened; infolded; sacculated; metaplastic	Thickened; infolded; sacculated; no metaplasia
Basement membrane	Wide; hyaline	Wide; hyaline	Wide; hyaline	Wide; hyaline	Wide; hyaline	Wide; hyaline	Wide; hyaline
Submucosa	Thickened; eosinophils	Thickened; eosinophils	Thickened; eosinophils	Thickened; eosinophils	Thickened; eosinophils	Thickened; eosinophils	Thickened; eosinophils
Musculature	Hypertrophied	Hypertrophied	Hypertrophied	Hypertrophied	Hypertrophied	Hypertrophied	Hypertrophied
Mucous glands	Numerous; distended; degenerated	Few; slight degenerative changes	Numerous; distended; degenerated	Numerous; distended; degenerated	.....	.....	Numerous; distended; degenerated
Cartilage	.....	Degeneration; fibrosis	Normal	Slight granular degeneration	.....	.....	Fibrous degeneration
Alveoli	Dilated, ... ruptured	Dilated, ruptured	Dilated, ruptured	Dilated, ruptured	Dilated, ruptured	Dilated, ruptured; slight bronchopneumonia	Dilated, ruptured, atelectatic; bronchopneumonia
Arterioles	Extreme thickening	Moderate to extreme thickening	Slight to extreme thickening	Slight thickening	Normal	Normal	Moderate thickening
Heart							
Myocardium	Slight edema	Normal	Slight edema	Normal	Normal	Normal	Slight edema

* C. L., Charcot-Leyden.

† C., Curschmann.

‡ This patient had ascites with 75 cc. of fluid.

degeneration varied from slight to great, and various degrees were noted in the same patient. Rarely was the basement membrane of the glands thickened like that of the bronchial mucosa. A widened submucosal layer was present in every instance, but it was not striking. In 3 instances degenerative changes in the bronchial cartilages were noted. Two patients had bronchopneumonia, and these were among the 4 who had had fever. In only 1 instance were areas of atelectasis present.

There was no important cardiac lesion in any of these patients. The hearts weighed from 200 to 365 Gm., the weight averaging 280 Gm. There was slight right-sided dilatation in 2 instances, but in none did the musculature of the right ventricle measure over 5 mm. in thickness. Occasionally slight relative hypertrophy of the right side was noted. In no case was there significant valvular or coronary arterial disease, and microscopic sections showed no evidence of myocardial scarring.

In 5 of the 6 instances in which the abdomen was examined the liver was congested.

In no case was a relevant, significant pathologic condition found in other parts of the body.

The pertinent postmortem observations are summarized in table 2.

COMMENT

This, then, is a group of cases of bronchial asthma in which the patients apparently died as a direct result of the disease. As Alexander [4] has stated, there is no single criterion for making a pathologic diagnosis of bronchial asthma. Every finding in asthma may be duplicated in some other pulmonary condition. However, asthma does produce a definite pathologic picture in the bronchial wall, described by Alexander as including: (1) a widened basement membrane, (2) infiltration of eosinophils, (3) a widened submucosal layer, (4) hypertrophy of muscle and (5) "degenerative" mucous glands. In these 7 cases all these criteria were met, with the exception of the degenerative bronchial glands in the 2 instances in which the microscopic sections showed none. In addition, 6 of the patients had the characteristic thick, tenacious, elastic bronchial exudate, and all had a clinical picture consistent with asthma.

A few other microscopic lesions may be briefly discussed. Areas of atelectasis, as Huber [5] pointed out, are a significant finding in that they indicate complete and persistent bronchial occlusion. Atelectasis has been infrequently mentioned in the literature and occurred in only 1 of this group of cases. As Huber stated, these areas are frequently sites

4. Alexander, H. L., in discussion on Michael, P. P., and Rowe, A. H.: Pathology of Two Fatal Cases of Bronchial Asthma, J. Allergy **6**:169 (Jan.) 1935.

5. Huber, H. L., in discussion on Michael, P. P., and Rowe, A. H.: Pathology of Two Fatal Cases of Bronchial Asthma, J. Allergy **6**:170 (Jan.) 1935.

TABLE 3.—*Effect of Drugs*

	A. D.	L. McD.	B. B.	I. B.	R. M.	E. K.	G. C.
Previous response to epinephrine..	Relief	Relief	Relief	Reaction	Relief	Relief	Not used
Dosage in hospital.....	Frequent; 0.1 to 1.0 cc.	Frequent; 0.5 to 0.8 cc.	Very frequent; 0.3 to 0.5 cc.	5 injections; 0.1 to 0.3 cc.	Very frequent; 0.5 cc.	2 injections; 1.0 cc.	2 injections; 0.5 to 1.0 cc.
Response.....	Diminishing relief	Diminishing relief	First relief, then failure	Slight	Diminishing relief	None	None
Atropine, dosage.....	0.25 mg.; 20 injections in 2 months	0.25 to 0.3 mg.; 64 injections in 21 days	None used	0.3 to 0.5 mg.; 3 injections in 2 days	0.3 to 0.6 mg.; 2 injections in 9 days	0.5 mg.; 1 injection in 3 days	None used
Morphine, frequency of use.....	10 (10 days)	Almost to addiction	6 (6 days)	2 (2 days)	1 injection	None used	None used
Last dose, time ante mortem.....	9 hours	7 days	3 days	30 hours	9½ hours	.....	.....
Digitalis, dose.....	4.1 Gm. (13 days); digitalization	2.7 Gm. (14 days); Nausea; stopped 4 days a. m.*	0.9 Gm. (9 days); previous digitalization	7 minims q. 5 h.; none last 2 days	0.3 Gm. (1 day); begun 1 day a. m.	None used	0.6 Gm. digitalis glucoside; last injection 1½ hours a. m.

* A. m. indicates ante mortem.

for the subsequent development of pneumonia, and in several atelectatic areas in the lungs of our patient such a change had occurred.

Another interesting observation is the finding of Charcot-Leyden crystals in the bronchial lumen. This occurred in 4 instances, and in 2 the crystals were large and abundant. This is apparently a rare occurrence, since a review of the descriptions of microscopic sections in the reported cases disclosed only 6, or possibly 7, instances in which the crystals had been seen. The possible seventh instance was described by Waldbott in the report of the case of an infant who had "some small spindle-shaped cells suggesting spores of fungi" in one of the bronchi.[6] The crystals have the shape of two elongated hexagonal pyramids placed base to base (fig. 3).[7] Typical crystals within the lumen of a bronchus were reported by Berkart[8] in 1889, by Mönckeberg[9] in 1909, by Marchand[10] in 1916, by Dehner[11] in 1927, by Kountz and Alexander[12] in 1928 and by Antoniazzi[13] in 1933.

The treatment of status asthmaticus is difficult, and the value of the therapeutic agents is uncertain. In this group each patient received a wide variety of drugs. Because four of these are of particular interest, the details of their administration are indicated in table 3.

It will be noted that 5 of the patients had previously obtained relief from epinephrine, 1 patient claimed to have had a severe reaction on the only occasion on which she had employed it and 1 patient allegedly had never used it. In the terminal illness the decreasing efficacy of epinephrine in relieving the symptoms was an important observation, for this made treatment extremely difficult. It is to be noted that the patient who had never previously received epinephrine gained no relief whatever from two injections. This suggests that a tolerance due to repeated administration of epinephrine may not be the reason for the patient's final failure to respond.

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6. Waldbott, G. L.: Pathologic Changes in Asthmatic Infants, Am. J. Dis. Child. **49**:1531-1539 (June) 1935.

7. Thompson, J. H., and Paddock, F. K.: The Significance of Charcot-Leyden Crystals, New England J. Med. **223**:936-939 (Dec.) 1940.

8. Berkart, J. I. B.: Bronchial Asthma, London, J. & A. Churchill, 1889; cited by Huber, H. L., and Koessler, K. K.: The Pathology of Bronchial Asthma, Arch. Int. Med. **30**:689-760 (Dec.) 1922.

9. Mönckeberg: Zur pathologischen Anatomie des Bronchialasthmas, Verhandl. d. deutsch. path. Gesellsch. **14**:173, 1909.

10. Marchand, F.: Beitrag zur Pathologie und pathologischen Anatomie des Bronchialasthmas, Beitr. z. path. Anat. u. z. allg. Path. **61**:251, 1916.

11. Dehner, W.: Beitrag zur Pathologie des Asthma bronchiale, Klin. Wchnschr. **6**:1412-1417 (July 23) 1927.

12. Kountz, W. B., and Alexander, H. L.: Death from Bronchial Asthma: Report of Three Cases, Arch. Path. **5**:1003-1019 (June) 1928.

13. Antoniazzi, E.: Un caso mortale di asma bronchiale, con autopsia, Policlinico (sez. med.) **40**:345-359 (June) 1933.

Atropine was frequently employed in this series. Thieme and Sheldon [1] stated that atropine may have an untoward effect in cases of asthma by thickening the bronchial secretion, thus making it more difficult to raise the mucus plugs. In this connection it is noteworthy that the patient with the best developed Curschmann's spirals (twisted, compact, concentrated bronchial plugs) was the patient who had received twenty injections of atropine during her terminal illness. On the other hand, the 1 patient who had received more atropine than that had little mucus in the bronchial tree.

Many authorities [14] have expressed the belief that morphine is contraindicated in status asthmaticus because it is a respiratory depressant. Most of the patients in this series received it, but a correlation between the time of death and the time of the last injection of morphine is not demonstrable. The last injection was administered nine, nine and a half and twenty hours and three and seven days, respectively, before death to the 5 patients receiving this drug.

Vaughan [14a] has suggested that digitalis may be a dangerous drug in treatment of asthma. In support of this contention he cited the work of Wearn to the effect that digitalis reduces the output of the normal heart. He stated that he had seen a patient in status asthmaticus die shortly after digitalization, apparently as a result of the treatment. The patients in this series had essentially normal hearts except for occasional slight relative right-sided hypertrophy. Most of them received digitalis, and at least 3 were digitalized, but there is no correlation between the time of death and either the time of administration of digitalis or the amount given. On the basis of these data no conclusions can be drawn as to whether digitalis is harmful.

A partial explanation of the signs of cardiac failure occasionally observed was suggested by the remarkable gross appearance of the lungs in situ. As the chest plate was removed the lungs bulged out of their respective pleural cavities and over the mediastinum as if they had been confined under pressure. This must indicate at least a decreased negative pressure in the thorax and probably a positive pressure, since normally the lungs collapse as atmospheric pressure is established in the pleural space.

In support of such an interpretation are the observations of Smith, Harter and Alexander [15] on anaphylactic shock in guinea pigs. With the onset of severe anaphylaxis the intrapleural pressure usually rose, after a

14. (*a*) Vaughan, W. T.: Practice of Allergy, St. Louis, C. V. Mosby Company, 1939. (*b*) Rowe, A. H.: Clinical Allergy, Philadelphia, Lea & Febiger, 1937. (*c*) Thieme and Sheldon.[1]

15. Smith, F. R.; Harter, J. S., and Alexander, H. L.: Observations on Circulation of Guinea Pigs During Bronchospasm, Proc. Soc. Exper. Biol. & Med. **25**:803 (June) 1928.

preliminary fall in 1 case to more than + 3 mm. of water from an initial level of — 13 mm. The pressure within the right auricle gradually rose from + 5 to + 70 mm. of water. The measurements tended to return to normal shortly before death. Though the change in intrapleural pressure was far from sufficient to give the effect of a tamponade, still it appears that in severe asthma the return of venous blood to the heart may be impeded. This may be a partial explanation of the frequently observed tachycardia.

In further support of this hypothesis are the observations of Alexander, Luten and Kountz,[17] who reported increased venous pressure in 31 of 39 asthmatic patients. The method used gave readings of 5.0 to 5.5 cm. of water in normal persons. In 31 of the 39 asthmatic patients the venous pressure ranged from 6.0 to 13.5 cm. These observations were usually made between attacks. During an attack the venous pressure was found to be elevated still further, though no figures were given. In the same group of patients there was no electrocardiographic evidence of right ventricular preponderance (1 exception) and usually no increase in the size of the heart. This appears to be additional evidence that in asthma the return of venous blood is impeded.

The similarity to the pressure relationships in constrictive pericarditis is obvious, though in asthma, of course, the signs are never so distinct.

It seems therefore not unreasonable to conclude that in severe status asthmaticus signs of right ventricular failure may sometimes appear and may be due entirely to extracardiac factors.

SUMMARY

The clinical and autopsy observations in the cases of 7 patients who died of bronchial asthma are reported.

The relationship between status asthmaticus and the signs of right-sided heart failure is discussed.

16. Footnote deleted by author.

17. Alexander, H. L.; Luten, D., and Kountz, W. B.: Effects on Heart of Long-Standing Bronchial Asthma, J. A. M. A. **88**:882-884 (March 19) 1927.

Progress in Internal Medicine

DISEASES OF NUTRITION

REVIEW OF CERTAIN RECENT CONTRIBUTIONS

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Within the past year vitamins again have been the main attraction in the study of nutrition. More and more evidence has accumulated to indicate that vitamins form an integral part of the enzyme system of the body and that clinical deficiencies of vitamins are usually multiple rather than singular. In addition, the broad aspect of national defense has again brought forward many timely discussions of national standards of nutrition and the controversial issue of fortification of foods. These general advances have been supplemented by numerous experimental and chemical contributions to knowledge of each separate vitamin, but space allows detailed discussion only of those of the greatest clinical significance.

VITAMIN A

Chemical and Physiologic Properties.—It appears from recent reports that the chemical structure of vitamin (A_1) is closely related to that of vitamin A_2. Most observations are in accord with the view that vitamin A_2 contains the same number of carbon atoms as vitamin A but differs in that it has one additional conjugated double bond.[1] For the first time a fatty acid ester of vitamin A and also an alcohol of vitamin A were prepared in crystalline form.[2]

Absorption studies on rats indicate that esters of vitamin A behave in the intestinal tract as do the esters of other fatty acids. Apparently they are hydrolyzed by the enzymes present, and during the height of absorption the vitamin exists in the intestinal wall chiefly as an alcohol. These facts, of course, argue against a major role for vitamin A in the

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1. Gray, E. LeB., and Cawley, J. D.: The Influence of Structure on the Elimination Maximum: I. The Structure of Vitamin A_2, J. Biol. Chem. **143**:397-401 (June) 1940.

2. Baxter, J. G., and Robeson, C. D.: Crystalline Vitamin A Palmitate and Vitamin A Alcohol, Science **92**:203-204 (Aug. 30) 1940.

absorption of fat.[3] Absorption of vitamin A by the tissues appears to be exceptionally rapid when it is administered intravenously. Groth and Skurnik [4] could not detect vitamin A in the urine of rabbits or men saturated with vitamin A administered in this manner.

In an interesting study Clausen and his associates [5] reported that after oral or intravenous administration of alcohol to dogs a sharp rise in the vitamin A content of the serum occurred. Ethyl alcohol injected directly into the portal vein of 1 animal produced a marked rise in the vitamin A content of the blood in the hepatic vein. This work was confirmed by Pett,[6] who demonstrated that vitamin A is mobilized in the blood of human beings after injection of ethyl alcohol.

It has long been known that carotene can be converted into vitamin A in the body. It was suggested that this conversion takes place in the liver by means of an enzyme called "carotenase"; it is believed generally that the liver plays an important role in regulating the distribution of vitamin A throughout the body. In experiments on rats which were given injections of carotene in oil and killed at different times, With [7] observed that the conversion of carotene to vitamin A took place almost entirely in the liver; this process began about two hours after an injection of carotene. Similar experiments carried out with vitamin A disclosed that thirty to sixty minutes after an injection of carotene vitamin A can be identified in the liver.

Perhaps the most interesting studies during the past year on the physiologic properties of vitamin A were made by Popper and his associates,[8] who studied the distribution of vitamin A in the body. This study was carried out by the fluorescence microscopic method, which permitted the visualization of vitamin A in tissue cells. Popper [8b] stated

3. Gray, E. L.; Morgareidge, K., and Cawley, J. D.: Intestinal Absorption of Vitamin A in the Normal Rat, J. Nutrition **20**:67-74 (July 10) 1940.

4. Groth, H., and Skurnik, L.: Intravenous Saturation with Vitamin-A, Acta med. Scandinav. **101**:333-337, 1939.

5. Clausen, S. W.; Baum, W. S.; McCoord, A. B.; Rydeen, J. O., and Breese, B. B.: Mobilization of Vitamin A from Its Stores in the Tissues by Ethyl Alcohol, Science **91**:318-319 (March 29) 1940.

6. Pett, L. B.: Mobilization of Vitamin A by Alcohol, Science **92**:63 (July 19) 1940.

7. With, T. K.: Investigations on Transformation of Carotene to Vitamin A in Liver of Rats, with Special Reference to Rate of Process, Nord. med. (Hospitalstid.) **3**:2901-2903 (Sept. 23) 1939.

8. (*a*) Popper, H.: Histological Demonstration of Vitamin A in the Human Liver by Means of Fluorescence Microscopy, Proc. Soc. Exper. Biol. & Med. **43**:234-236 (Feb.) 1940; (*b*) Vitamin A: The Distribution of Vitamin A in the Body, J. Mt. Sinai Hosp. **7**:119-132 (Sept.-Oct.) 1940. (*c*) Popper, H., and Greenburg, R.: Distribution of Vitamin A in the Rat as Studied by Fluorescence Microscopy, Am. J. Physiol. **129**:442 (May) 1940.

that five years ago von Querner [9] described fluorescent inclusions in the epithelial cells of the liver, the adrenal cortex and the pituitary gland, from which the fluorescence faded on irradiation. Popper maintained that the same fluorescence could be found in vitamin A concentrate and expressed the belief that this fluorescence of the tissue established the presence of vitamin A.

In ultraviolet light vitamin A has a characteristic green fluorescence which is used in the fluorescence microscopic method of demonstrating the presence of vitamin A in human and animal tissues.

Popper and his associates [8c] carried out studies first on experimental animals. It was found that the liver of a normal rat contained large amounts of the characteristic green fluorescent substance, but the liver of a rat deficient in vitamin A showed none. However, if the deficient rats were fed vitamin A the typical fluorescence became visible. Popper and his associates observed further that the fluorescent substance was not vitamin A alone but rather small lipoid droplets carrying the vitamin. In the intestine of the rat vitamin A could not be demonstrated, but after oral administration of vitamin A the fluorescence was seen in the lumen and within the wall of the duodenum and the upper portion of the jejunum.

It is interesting also that the retina of the rat contained considerable vitamin A in fine droplets and that even in the severe form of avitaminosis this material did not entirely disappear but seemed only to be reduced.[8c] In fact, vitamin A was present in the retina of rats which died with avitaminosis and ulceration of the cornea.

Vitamins A (A_1) and A_2 also could be differentiated by the fluoescence microscopic method. Vitamin A_2 gave a brown-red, slowly fading fluorescence, instead of the green fluorescence common for vitamin A. Carotene also presented a type of fluorescence different from that of vitamin A_1. When carotene was administered, the fluorescence characteristic of this compound appeared in the epithelial cells of the liver, the lungs and the kidneys. The intestine usually was free of the fluorescence characteristic of carotene after its administration. This observation led to the assumption that carotene is split in the endothelial cells of the intestine and especially in the Kupffer cells of the liver.

Popper with Greenburg also studied the distribution of vitamin A in human organs. It was found that the distribution of vitamin A in the human liver varied in amount even under normal conditions. In infants there was little storage of vitamin A in the liver, but in the fetus of about 5 months considerable amounts were present. Toward term these depots were reduced, and at birth only traces of vitamin A were distinct. Popper reported that it takes several years until the normal depots of

9. von Querner, F. R.: Der mikroskopische Nachweis von Vitamin A im animalen Gewebe. Zur Kenntnis der paraplasmatischen Leberzelleinschlusse: III, Klin. Wchnschr. **14**:1213-1217 (Aug. 24) 1935.

vitamin A of the adult level are reached. In cases of generalized hepatic damage the storage of vitamin A was reduced, and in cases of such diseases as acute hepatitis or chronic atrophy of the liver only traces of vitamin A could be found. In cases of decompensated cirrhosis the liver was nearly free of vitamin A.

Tissues of the adrenal glands and lactating breasts of human beings were rich in vitamin A, but tissues from apparently normal kidneys, inactive breasts and the brain and the epithelium of the cornea, bronchi and urinary tract were found to be free of the vitamin. By the fluorescence microscopic technic, vitamin A was found in tumors which originated from a vitamin A—carrying mother tissue only.

We have described this work in some detail because it is felt that the fluorescence microscopic method offers rather widespread possibilities and it is hoped that in the near future other investigators will attempt to repeat and extend these efforts.

Vitamin A Requirements of Man.—The definite minimal requirement of vitamin A of man is still unknown. In an extensive study, May and his associates [10] found that normal, well nourished infants and children have levels of vitamin A in their blood which range from 5.5 to 27.3 units.[10a] The levels of carotenoids in the blood of normal infants and children range from 3.1 to 75.7 units. These investigators observed that in patients with acute infections associated with fever the levels of both vitamin A and carotenoid in the blood were decreased. In such conditions, these low levels, however, should not be taken to indicate an inadequate intake of these substances or a depletion of their stores in the body.

Although most clinicians think that the average diet of the infant should be supplemented with vitamin A, Lewis and Haig [11] suggested that this is unnecessary. These authors reported that infants who received a diet low in vitamin A gained in weight just as well as, and were no more susceptible to infection than, those who received a diet high in vitamin A. From their observations it was concluded that 135 to 200 U. S. P. units of vitamin A, approximately 25 units per kilogram of body weight, covered the daily minimal requirement of vitamin A for the particular group of infants studied. They pointed out that because the average diet contains approximately twelve times as many

10. May, C. D.; Blackfan, K. D.; McCreary, J. F., and Allen, F. H., Jr.: Clinical Studies of Vitamin A in Infants and in Children, Am. J. Dis. Child. **59**: 1167-1184 (June) 1940.

10a. The values are expressed, not in U. S. P. units, but abstract units directly related to the galvanometer readings on the Evelyn colorimeter, as described by May (page 1170 [10]).

11. Lewis, J. M., and Haig, C.: Vitamin A Requirements in Infancy as Determined by Dark Adaptation, J. Pediat. **15**:812-823 (Dec.) 1939; correction, ibid. **16**:274 (Feb.) 1940.

units of vitamin A as the diet low in vitamin A which they used, there is a large margin of safety in the average infant's diet with respect to content of vitamin A.

In a further study of minimal requirements of vitamin A of normal adults, Booher and Callison [12] found that a daily intake of approximately 47 and 57 U. S. P. units, respectively, of vitamin A per kilogram of body weight was necessary for the maintenance of normal dark adaptation in 2 normal adults when the vitamin A in the diet was derived almost entirely from the carotene in cooked green peas. They observed that the utilization of the vitamin A of cooked peas was better than that of vitamin A of cooked spinach. They further demonstrated that administration of thiamine hydrochloride, riboflavin or dietary fats in large quantities in addition to carotene did not have beneficial effects on the utilization of carotene by these normal adults.

In a special article published by the Council on Foods of the American Medical Association, Dornbush and his associates [13] described a study of the content of carotene and vitamin A in milk marketed by eight large distributors in the Madison and the Milwaukee areas of Wisconsin. They observed that all milk had seasonal changes in content of both carotene and vitamin A; the seasonal changes in carotene were greater than those in vitamin A. Milk was fairly constant in vitamin potency per gram of butter fat. The market milk (mainly Holstein) supplied from January to April averaged 327 micrograms, or 1,088 U. S. P. units, of vitamin A per quart.

Methods of Measuring Vitamin A Deficiency.—Whether deficiency of vitamin A in man can be measured by testing adaptation to dark continues to be a controversial subject. Several groups of investigators [14] contended that this method is satisfactory for measuring deficiency of vitamin A in man. Steininger and Roberts,[15] however, contended that

12. Booher, L. E., and Callison, E. C.: The Minimum Vitamin-A Requirements of Normal Adults, the Utilization of Carotene as Affected by Certain Dietary Factors and Variations in Light Exposure, J. Nutrition **18**:459-471 (Nov.) 1939.

13. Dornbush, A. C.; Peterson, W. H., and Olson, F. R.: The Carotene and Vitamin A Content of Market Milks, J. A. M. A. **114**:1748-1751 (May 4) 1940.

14. Steele, E. J. P.: Effect of Vitamin-A Therapy Estimated by a Rapid Optical Test, Lancet **2**:205-206 (Aug. 17) 1940. Sheftel, A. G.: Dark Adaptation and Vitamin A Deficiency: A New Technic, Am. J. Clin. Path. **10**:168-175 (Feb.) 1940. Pett, L. B.: Vitamin A Deficiency: Its Prevalence and Importance as Shown by a New Test, J. Lab. & Clin. Med. **25**:149-160 (Nov.) 1939. Blanchard, E. L., and Harper, H. A.: Measurement of Vitamin A Status of Young Adults by the Dark Adaptation Technic, Arch. Int. Med. **66**:661-669 (Sept.) 1940. McDonald, R., and Adler, F. H.: Effect of Anoxemia on the Dark Adaptation of the Normal and of the Vitamin A-Deficient Subject, Arch. Ophth. **22**:980-988 (Dec.) 1939.

15. Steininger, G., and Roberts, L. J.: Biophotometer Test as an Index of Nutritional Status for Vitamin A, Arch. Int. Med. **64**:1170-1186 (Dec.) 1939.

although some relation exists between the biophotometric readings and vitamin A nutrition, the relation is not close enough to warrant the use of the test as a means of diagnosing subclinical deficiency of vitamin A. These authors pointed out that the method was time consuming and that for this reason its routine clinical use was practically excluded. Harris and Abbasy [16] expressed a similar opinion, and Isaacs and her associates [17] emphasized that significance should not be attached to minor fluctuations in dark adaptation in terms of deficiency of vitamin A unless statistical methods are used to test the reliability of the differences. It is true that a majority of workers have concluded that the study of dark adaptation can be used as a test for deficiency of vitamin A, but Thomson and his associates [18] pointed out that until differences in technic and interpretation of results have been resolved it will be impossible to ascertain how far recorded observations represent physiologic facts.

In a study of children, Baum and McCoord [19] did not find any correlation between biophotometric readings and the content of vitamin A in the blood of 98 untrained subjects. They pointed out that a single reading on the biophotometer was not of value in estimating deficiency of vitamin A and that readings obtained by frequent testing with the biophotometer did not correlate with the vitamin A value of blood of these particular subjects. Several other groups of investigators studied the level of vitamin A in the blood of normal adults.[20] Steininger and her associates [20a] studied the level of vitamin A in the blood of 4 normal

16. Harris, L. J., and Abbasy, M. A.: The Dark-Adaptation Test: Its Reliability as a Test for Vitamin-A Deficiency, Lancet **2**:1299-1305 (Dec. 23) and 1355-1359 (Dec. 30) 1939.

17. Isaacs, B. L.; Jung, F. T., and Ivy, A. C.: Clinical Studies of Vitamin A Deficiency: Biophotometer and Adaptometer (Hecht) Studies on Normal Adults and on Persons in Whom an Attempt Was Made to Produce Vitamin A Deficiency, Arch. Ophth. **24**:698-721 (Oct.) 1940.

18. Thomson, A. M.; Griffith, H. D.; Mutch, J. R.; Lubbock, D. M.; Owen, E. C., and Logaras, G.: A Study of Diet in Relation to Health: Dark Adaptation as an Index of Adequate Vitamin A Intake; III. The Relation of Diet to Rate and Extent of Dark Adaptation, Brit. J. Ophth. **23**:697-723 (Nov.) 1939.

19. Baum, W. S., and McCoord, A. B.: The Relationship Between Biophotometer Tests and the Vitamin A Content of the Blood of Children, J. Pediat. **16**:409-418 (April) 1940.

20. (*a*) Steininger, G.; Roberts, L. J., and Brenner, S.: Vitamin A in the Blood of Normal Adults: The Effect of a Depletion Diet on Blood Values and Biophotometer Readings, J. A. M. A. **113**:2381-2387 (Dec. 30) 1939. (*b*) Pett, L. B., and LePage, G. A.: Vitamin A Deficiency: III. Blood Analysis Correlated with a Visual Test, J. Biol. Chem. **132**:585-593 (Feb.) 1940. (*c*) French, R. B.: Assay of Vitamin A with the Photoelectric Colorimeter, Indust. & Engin. Chem. (Analyt. Ed.) **12**:351-352 (June) 1940. (*d*) Kimble, M. S.: The Photocolorimetric Determination of Vitamin A and Carotene in Human Plasma, J. Lab. & Clin. Med. **24**:1055-1065 (July) 1939.

subjects who lived on a vitamin A depletion diet for two to four months. The results suggested that although the amount of vitamin A in the blood of fasting persons is dependent on the amount contained in the diet, evidence as to whether the determinations of vitamin A in the blood are of value in judging the nutritional status of man is still contradictory.

From these studies it can be judged that the methods for measuring vitamin A deficiency in man are still somewhat unreliable and further investigation is needed. Some chemists still doubt whether the small quantities of vitamin A present in the blood stream of man can be measured by the chemical methods now available. Others feel that a satisfactory chemical method cannot be developed until the capacity of the body for storing vitamin A can be estimated to some degree.

Role of Vitamin A in Health and Disease.—Inability to absorb vitamin A in a normal manner exists in cases of celiac disease,[21] catarrhal jaundice [22] and various diseases of the liver in which the flow of bile is disrupted. Breese, McCoord and Salah [23] stressed the importance of considering this physiologic fact in the treatment of these diseases.

Straumfjord [24] concluded that vernix caseosa is a manifestation of a deficiency of vitamin A in the newborn and that it represents disturbances in cornification analogous to the cutaneous changes accompanying keratomalacia and other manifestations of deficiency of vitamin A. This author administered 50,000 to 100,000 or more U. S. P. units of vitamin A daily to 25 pregnant mothers for six months or more. Twenty-one babies born to these 25 mothers had little or no vernix; 4 babies had moderate or much vernix. To another group of 29 mothers, less than 50,000 U. S. P. units of vitamin A was administered daily for six months or more. Fourteen of the babies born to this group of mothers had little or no vernix, and 15 were born with moderate or much vernix. Lehman and Rapaport [25] described cutaneous manifestations of deficiency of vitamin A in children. These authors obtained maximal improvement of the skin with daily doses of 100,000 to 300,000 international units of vitamin A given over two or three months. They expressed the belief that keratosis pilaris, ichthyosis follicularis and other synonyms were descriptive terms for the cutaneous manifestations of

21. Breese, B. B., Jr., and McCoord, A. B.: Vitamin A Absorption in Celiac Disease, J. Pediat. **15**:183-196 (Aug.) 1939.

22. (*a*) Breese, B. B., and McCoord, A. B.: Vitamin A Absorption in Catarrhal Jaundice, J. Pediat. **16**:139-145 (Feb.) 1940. (*b*) Salah, M.: Vitamin A Deficiency in Jaundice, J. Egyptian M. A. **23**:153-161 (March) 1940.

23. Breese and McCoord (footnotes 21 and *22a*). Salah.[22b]

24. Straumfjord, J. V.: Vernix Caseosa: A Manifestation of Vitamin A Deficiency; a Preliminary Report, West. J. Surg. **48**:341-351 (June) 1940.

25. Lehman, E., and Rapaport, H. G.: Cutaneous Manifestations of Vitamin A Deficiency in Children, J. A. M. A. **114**:386-393 (Feb. 3) 1940.

deficiency of vitamin A. Other investigators [26] suggested that vitamin A exerted a favorable effect on the nutrition of the intestinal lining and was, therefore, of some use in the treatment of typhoid among children.

Accumulated evidence continues to indicate that the liver plays a major role in the metabolism of vitamin A. Jensen and With [27] and others [28] found extremely low concentrations of vitamin A and carotene in the livers of the patients who had cirrhosis. Low concentrations of vitamin A in the liver also have been observed among patients with dysentery,[28] tuberculosis [29] or various hepatic disorders.[30]

Brazer and Curtis [31] carried out a rather extensive study of the metabolism of vitamin A in cases of juvenile diabetes mellitus. These investigators observed 20 patients who had diabetes mellitus and normal ocular fundi but who, as determined by biophotometric readings, had a suggestive deficiency of vitamin A in spite of the fact that the levels of carotene in the blood were above normal. Three of these 20 patients gave a history of night blindness, and 9 showed clinical evidence of mild deficiency of vitamin A as exemplified by various cutaneous changes. An increase in the already high values for carotene in the blood occurred after administration of large doses of carotene, but the biophotometric readings failed to indicate improvement. Large amounts of vitamin A given over a similar period, however, produced significant improvement as measured by the biophotometric readings. It was observed that if the large doses of vitamin A were removed from the diet of patients whose biophotometric readings were held at normal or nearly normal by these doses, an immediate relapse of the biophotometric readings to subnormal value occurred. Levels of carotene in the blood in 90 per cent of these 20 patients with diabetes mellitus were above normal. Administration of 60,000 U. S. P. units of vitamin A in the form of crystalline carotene dissolved in a vegetable oil given for fourteen days

26. Giraud, P., and Vallette, A.: Essai de traitement de la fievre typhoïde chez l'enfant par la vitamine A, Arch. de méd. d. enf. **42**:691-697 (Nov.-Dec.) 1939; abstracted, J. A. M. A. **114**:832-833 (March 2) 1940.

27. Jensen, H. B., and With, T. K.: Vitamin A and the Carotenoids in the Liver of Mammals, Birds, Reptiles and Man with Particular Regard to the Intensity of the Ultraviolet Absorption and the Carr-Price Reaction of Vitamin A, Biochem. J. **33**:1771-1786 (Nov.) 1939.

28. Woo, T. T., and Chu, F. T.: The Vitamin A Content of the Livers of Chinese Infants, Children and Adults, Chinese J. Physiol. **15**:83-100 (Jan. 30) 1940.

29. Crimm, P. D., and Short, D. M.: Vitamin A Content of the Human Liver in Tuberculosis, Ann. Int. Med. **13**:61-63 (July) 1939.

30. Wohl, M. G., and Feldman, J. B.: Vitamin A Deficiency in Disease of the Liver: Its Detection by Dark-Adaptation Method, J. Lab. & Clin. Med. **25**: 485-494 (Feb.) 1940.

31. Brazer, J. G., and Curtis, A. C.: Vitamin A Deficiency in Diabetes Mellitus, Arch. Int. Med. **65**:90-105 (Jan.) 1940.

did not affect light adaptation of the patients with this condition, but the daily administration of 60,000 U. S. P. units of vitamin A in the form of concentrated fish liver oil increased their light adaptation in three to twenty-one days. The authors suggested that poor adaptation to light among patients who have juvenile diabetes mellitus might result from an inability to convert carotene into vitamin A.

The uses and requirements of vitamin A in a variety of other diseases have also been reported during the past year. Hall and his associates [32] did not observe obvious clinical improvements after administration of large doses of vitamin A to patients who had rheumatoid arthritis. In a preliminary report, asthenopia was considered the result of deficiency of vitamin A.[33] One author suggested the local application of vitamin A in cases of corneal inflammation;[34] another author [35] demonstrated experimentally the lack of evidence of the local action of vitamin A.

THIAMINE (VITAMIN B_1)

Chemical and Physiologic Properties.—Much work on the relation of thiamine to the metabolism of pyruvic acid was carried out during the past year. Platt and Lu [36] observed that in human subjects elevated levels of blood pyruvate were often associated with deficiency of thiamine. They also found that the initial level of blood pyruvate was a definite indication of the degree of deficiency in cases of beriberi. In such cases several hours were required after the administration of thiamine before the accumulated pyruvate was removed from the blood. Only 5 mg. of thiamine hydrochloride was needed to restore the normal metabolism of pyruvic acid in cases of severe beriberi. Further studies by these authors [37] demonstrated that light muscular work by patients who were deficient in thiamine was followed by an increased level of pyruvate in the blood and increased urinary excretion of pyruvic acid. Values for blood pyruvate as high as those found in cases of fulminating beriberi

32. Hall, M. G.; Bayles, T. B., and Soutter, P.: Vitamin A Requirements in Rheumatoid Arthritis, New England J. Med. **223**:92-96 (July 18) 1940.

33. Cordes, F. C., and Harrington, D. O.: Astenopia Due to Vitamin-A Deficiency, Am. J. Ophth. **22**:1343-1354 (Dec.) 1939.

34. de Grósz, S.: Local Use of Vitamin A Preparations in Ophthalmic Practice, Arch. Ophth. **22**:727-734 (Nov.) 1939.

35. de Rötth, A.: Local Action of Oils Containing Vitamin A: Experimental Contribution, Arch. Ophth. **24**:281-291 (Aug.) 1940.

36. Platt, B. S., and Lu, G. D.: Studies on the Metabolism of Pyruvic Acid in Normal and Vitamin B_1 Deficiency States: IV. The Accumulation of Pyruvic Acid and Other Compounds in Beri-Beri and the Effect of Vitamin B_1, Biochem. J. **33**:1525-1537 (Oct.) 1939.

37. Lu, G. D., and Platt, B. S.: Studies on the Metabolism of Pyruvic Acid in Normal and Vitamin B_1 Deficiency States: V. The Effect of Exercises on Blood Pyruvate in Vitamin B_1 Deficiency in Man, Biochem. J. **33**:1538-1543 (Oct.) 1939.

were occasionally attained after exercise by deficient patients, but among normal patients the blood pyruvate usually returned to a normal level after half an hour of rest. These authors [37] expressed the belief that the increase in bisulfite-binding substances in patients who had beriberi was greater than could be accounted for by pyruvic acid alone, because the content of pyruvic acid in the blood frequently can be restored to normal while at the same time only a slight reduction in the bisulfite-binding substances takes place. On the other hand, Robinson and his associates [38] found the bisulfite-binding substances increased in 7 of 26 cases in which excretion of thiamine in the urine was normal. In 7 of 16 cases in which the concentration of thiamine in the urine was indicative of thiamine subnutrition the levels of bisulfite-binding substances were normal. One patient was on a diet inadequate in thiamine for twenty-two days; in this period urinary excretion of thiamine decreased to a low level, yet there was no increase of the bisulfite-binding substances in the blood. From these investigations the authors concluded that the determination of the bisulfite-binding substances in the blood lacked sensitivity as a means of detecting latent or mild forms of deficiency of thiamine.

Another interesting phenomenon resulting from the administration of thiamine to experimental animals was reported by Engel and Phillips.[39] These authors demonstrated that when thiamine hydrochloride was administered to chicks or rats deficient in thiamine hydropic degeneration and fatty metamorphosis occurred in the parenchyma of the hepatic cells. This histologic reaction in the liver of chicks was not prevented by administration of choline or by diets high in casein. Desiccated thyroid, however, was effective in preventing histologic reactions in the livers of both animals. Chemical analysis of the livers of these animals disclosed an increase in total fat, glycogen and moisture, but no change in the content of phospholipid or protein. It was observed also that hepatic degeneration did not occur after administration of thiamine if the animals were not allowed to eat.

Evidence was presented which suggested that animals deficient in calcium were incapable of utilizing the thiamine available in their diet.[40] Sinclair [41] found thiamine in extremely small amounts in cerebrospinal fluid but expressed the belief that estimation of the substance in the

38. Robinson, W. D; Melnick, D., and Field, H., Jr.: Correlation Between the Concentration of Bisulphite Binding Substances in the Blood and the Urinary Thiamin Excretion, J. Clin. Investigation **19**:483-488 (May) 1940.

39. Engel, R. W., and Phillips, P. H.: Fatty Livers as a Result of Thiamin Administration and the Vitamin B_1 Deficiency of the Rat and the Chick, J. Nutrition **18**:329-338 (Oct. 10) 1939.

40. Badger, L. F., and Masunaga, E.: Possible Relation of Calcium Deficiency to the Utilization of Vitamin B_1, Pub. Health Rep. **54**:1775-1779 (Sept. 29) 1939.

41. Sinclair, H. M.: The Estimation of Vitamin B_1 in Cerebrospinal Fluid, Biochem. J. **33**:1816-1821 (Nov.) 1939.

fluid was useless for clinical purposes. The highest values were obtained in cases of meningitis and of cerebral abscess.

Methods of Measuring Vitamin B_1 and the Requirement of the Normal Person.—Some excellent studies were carried out in this particular field. Melnick and his associates [42] observed that a considerable part of the thiamine hydrochloride which was taken into the stomach of a fasting person was destroyed prior to absorption and that if it was given parenterally, most of it was excreted in the urine in the first four hours. Women were found to excrete much less thiamine than men, and the volume of urine appeared to be an insignificant factor in governing the urinary excretion of thiamine. Ninety micrograms for males and 60 micrograms for females were established as the normal minimal values of thiamine excreted in a twenty-four hour sample of urine. Values below these levels were considered to be evidence of a possible deficiency of thiamine. The thiamine in the urine of a person subsisting on a diet deficient in vitamin B_1 for thirty-two days for investigative purposes decreased from normal to a level characteristic of avitaminosis. Although low urinary excretion is significant, it apparently does not indicate a deficiency in the vitamin, as it may mean that the patient's diet has recently been deficient in thiamine. However, if the patient was given a normal diet a few days prior to and during the day of the test and still a small amount of the vitamin was excreted, deficiency was considered to be present. It was observed that two weeks were required before urinary excretion of thiamine returned to the basal level after the patient had been taking a deficient diet. Borson [43] used a modification of the thiochrome reaction for the chemical assay of thiamine in the urine and observed that normal persons excreted 100 to 300 micrograms daily. He expressed agreement with Melnick and his associates [42] that the efficiency of utilization of thiamine depended in part on the degrees of absorption and excretion; that is, large doses administered orally are absorbed incompletely and large doses administered parenterally are excreted rapidly. Therefore, small frequent doses are utilized most efficiently.

Several other investigators [44] found somewhat similar levels of excretion of thiamine among normal subjects. In some cases of diffuse

42. Melnick, D.; Field, H., Jr., and Robinson, W. D.: A Quantitative Chemical Study of the Urinary Excretion of Thiamine by Normal Individuals, J. Nutrition **18**:593-610 (Dec.) 1939.

43. Borson, H. J.: Clinical Application of the Thiochrome Reaction in the Study of Thiamin (Vitamin B_1) Deficiency, Ann. Int. Med. **14**:1-27 (July) 1940.

44. Wang, Y. L., and Harris, L. J.: Methods for Assessing the Level of Nutrition of the Human Subject: Estimation of Vitamin B_1 in Urine by the Thiochrome Test, Biochem. J. **33**:1356-1369 (Aug.) 1939. Sinclair, H. M.: The Estimation of Vitamin B_1 in Blood: II. A Further Modification of Meiklejohn's Method, ibid. **33**:2027-2036 (Dec.) 1939. Wright, M. D., and Baker, A. Z.: Vitamin B_1 Excretion on a Varied Intake, J. Hyg. **39**:638-642 (Nov.) 1939.

hepatic damage,[43] excretion of thiamine was much higher than is considered normal, but in cases of clinical diabetes mellitus a particular unsaturation of thiamine was not observed.[45] In cases of "alcoholic beriberi," also, abnormally low excretion of thiamine in the urine was observed.[46] In a study carried out in China, Hou and Yang [47] did not observe excretion of thiamine in the urine of patients who had beriberi. On the other hand, Pannekoek-Westenburg and van Veen [48] observed divergent values for thiamine in the blood in 19 cases of manifest beriberi. In 9 of these 19 cases values were low, but in some of the cases of severe beriberi they were normal. The blood levels of thiamine in a majority of 165 cases of nutritional edema and in all but 2 of 19 cases of polyneuritis of various origins were within the normal range. On the basis of these rather extensive observations, the authors concluded that the determination of thiamine in the blood is not yet of great practical importance. Goodhart and Sinclair [49] presented evidence which demonstrated that the amount of cocarboxylase in the blood as determined by chemical methods varied directly with the amount of total thiamine as determined by biologic methods and also with the degree of saturation of the tissues with the vitamin. In most clinical cases, the determination of the amount of cocarboxylase in the blood provided a rapid and reliable method of estimating the degree of saturation of the tissues with the vitamin.

Many other methods for the chemical determination of thiamine in the urine of man also were described.[50]

45. Pollack, H.; Dolger, H.; Ellenberg, M., and Cohen, S.: A Test Proposed to Measure Vitamin B_1 Saturation in Humans, Proc. Soc. Exper. Biol. & Med. **44**:98-100 (May) 1940.

46. Robinson, W. D.; Melnick, D., and Field, H., Jr.: Urinary Excretion of Thiamin in Clinical Cases and the Value of Such Analyses in the Diagnosis of Thiamin Deficiency, J. Clin. Investigation **19**:399-408 (March) 1940.

47. Hou, H. C., and Yang, E. F.: Observations on Vitamin B_1 Metabolism: I. Urinary Excretion by Normal Individuals and Beriberi Patients, Chinese J. Physiol. **14**:269-282 (Sept. 30) 1939.

48. Pannekoek-Westenburg, S. J. E., and van Veen, A. G.: Vitamin B_1 Content of Blood in Healthy Persons and Patients, Geneesk. tijdschr. v. Nederl.-Indië **80**: 1773-1820 (July 23) 1940; abstracted, J. A. M. A. **115**:1494 (Oct. 26) 1940.

49. Goodhart, R., and Sinclair, H. M.: Deficiency of Vitamin B_1 in Man as Determined by the Blood Cocarboxylase, J. Biol. Chem. **132**:11-21 (Jan.) 1940.

50. Pollack, H.; Dolger, H.; Ellenberg, M., and Cohen, S.: Test for the Rapid Estimation of Vitamin B_1 Saturation, J. Clin. Investigation **19**:771 (Sept.) 1940. Emmett, A. D.; Peacock, G., and Brown, R. A.: Chemical Determination of Thiamine by a Modification of the Melnick-Field Method, J. Biol. Chem. **135**: 131-138 (Aug.) 1940. Hills, G. M.: The Thiochrome Test for Aneurin (Vitamin B_1) in Urine as an Index of Nutritional Level, Biochem. J. **33**:1966-1979 (Dec.) 1939. Ferrebee, J. W., and Carden, G. A.: A Procedure for the Routine Determination of Vitamin B_1 in Urine, J. Lab. & Clin. Med. **25**:1320-1324 (Sept.) 1940. Melnick, D., and Field, H., Jr.: Chemical Determination, Stability, and Form of Thiamine in Urine, J. Biol. Chem. **130**:97-107 (Sept.) 1939.

Clinical Deficiencies of Thiamine.—Deficiency of thiamine has been induced in human beings by several investigators.[51] The symptoms of deficiency usually appear in three to six weeks. Chemical evidence of the deficiency usually is characterized by aching of the muscles of the calf and achilles tendons and by paresthesia of the lower extremities. Marked fatigue, lassitude, anorexia, burning of the feet, dyspnea on exertion, palpitation, constipation, loss of weight and absence, of, or decrease in, gastric acidity are also noted. Abnormality is evidenced in the electrocardiograms. In this direction the study by Elsom and his associates [51d] is particularly interesting. These authors reported a study made on 1 person who voluntarily consumed for four months a constant diet deficient only in the vitamin B complex. In general, clinical metabolic changes were evident after five weeks, but they were not striking until the end of eight weeks. Edema appeared early in the deficiency, and loss of weight occurred late. The clinical and metabolic changes responded in part to thiamine hydrochloride, were influenced only slightly by thiamine hydrochloride and riboflavin and were relieved by the administration of brewers' yeast. The main changes were in the carbohydrate metabolism. During the deficiency, the blood sugar failed to return to normal limits within three to four hours after ingestion of dextrose. This effect disappeared only after administration of yeast. The bisulfite-binding power of the blood during deficiency was not increased, but after ingestion of dextrose the values for pyruvic acid were elevated and remained high for four hours or until thiamine hydrochloride was administered. The value for lactic acid in the blood was elevated during the deficiency and also returned to normal after the administration of thiamine hydrochloride.

Considerable investigation of the effect of thiamine in treatment of various neurologic disorders has continued, but the exact relation of thiamine to the metabolism of nerve tissue is still incompletely understood. This enigma is again voiced in an interesting article by Meiklejohn,[52] who asked, "Is thiamine the antineuritic vitamin?" From the

51. (*a*) Jolliffe, N.; Goodhart, R.; Gennis, J., and Cline, J. K.: The Experimental Production of Vitamin B_1 Deficiency in Normal Subjects: The Dependence of the Urinary Excretion of Thiamin on the Dietary Intake of Vitamin B_1, Am. J. M. Sc. **198**:198-211 (Aug.) 1939. (*b*) Williams, R. D.; Mason, H. L., and Smith, B. F.: Induced Vitamin B_1 Deficiency in Human Subjects, Proc. Staff Meet., Mayo Clin. **14**:787-793 (Dec. 13) 1939. (*c*) Williams, R. D.; Mason, H. L., and Wilder, R. M.: Experimental Vitamin B_1 Deficiency in Man, J. Nutrition (supp.) **19**:7 (June) 1940. (*d*) Elsom, K. O.; Lukens, F. D. W.; Montgomery, E. H., and Jonas, L.: Metabolic Disturbances in Experimental Human Vitamin B_1 Deficiency, J. Clin. Investigation **19**:153-161 (Jan.) 1940. (*e*) Melnick, Field and Robinson.[42]

52. Meiklejohn, A. P.: Is Thiamin the Antineuritic Vitamin? New England J. Med. **223**:265-273 (Aug. 22) 1940.

experimental side he pointed out that although it is possible that chronic, though partial, deficiency of thiamine may result in true polyneuritis, this does not appear to have been adequately demonstrated as yet. Furthermore, in his study he found no clear experimental evidence that true anatomic polyneuritis in animals can be cured by thiamine hydrochloride; for these and for other reasons, he concluded that so far as the evidence from the laboratory is concerned there is really no great justification for referring to thiamine as the antineuritic vitamin. Because the chemical identification of thiamine was not made until 1936, Meiklejohn [52] expressed the opinion that any article prior to this date in which specific claims were made for the etiologic relation of thiamine deficiency to nutritional polyneuritis cannot be cited without reservations. He correctly pointed out that the failure to appreciate this fact has led to several misrepresentations in recent articles. In a careful analysis of the literature since synthetic thiamine hydrochloride first became available for clinical use, Meiklejohn [52] found considerable lack of evidence concerning the use of thiamine hydrochloride in the treatment of polyneuritis. In most instances the composition and type of diet were not mentioned, and in many instances other members of the vitamin B complex were given. The possibility, therefore, of other dietary factors contributing to the observed response cannot be excluded. The evidence throughout seems to rest mainly on the relief by administration of thiamine hydrochloride of the symptoms of pain, weakness and inability to walk. In some unpublished data, Meiklejohn [52] has observed that thiamine hydrochloride without other medication may have a marked effect in the relief of muscular pain in tender calves occurring in cases of nutritional polyneuritis, but in the cases observed by him there was no associated improvement in the objective neurologic signs, and certainly the relief of the pain cannot be taken as evidence of improvement of the neuritis.

Meiklejohn [52] stated that polyneuritis associated with alcoholism, pregnancy and gastrointestinal disturbances is unquestionably due to nutritional deficiency and is in every way similar to the polyneuritis of oriental beriberi. But he commented as follows:

> When the literature of the last ten years is considered in perspective, the conclusion is inescapable that thiamin has never deserved the title of "antineuritic vitamin," and has not yet shown itself capable of filling completely the role that was formerly assigned to the hypothetical antiberiberi vitamin.
>
> The exact deficiency responsible for nutritional neuritis remains obscure. Quite probably it is usually a multiple deficiency involving several dietary factors, of which thiamin may be one. But there is still a possibility that the true antineuritic vitamin has yet to be discovered.

For these reasons he suggested, as did many others, that the treatment of polyneuritis should include "an ample and nutritious diet,

together with the administration of such preparations as yeast and crude liver extract to ensure an adequate supply of the entire vitamin B complex."

Aring and Spies [53] expressed the belief that the rapid relief of discomfort in neuritis of man probably results from the humoral nature of the vitamin, but that lack of thiamine alone is not the specific cause of nutritional neuritis. They recommended the parenteral administration of at least 50 to 100 mg. of thiamine hydrochloride each day, in several doses of 10 to 20 mg. each. It is their belief that if a response is not obtained in a week further treatment with thiamine hydrochloride will be unavailing. From a study based on 520 patients traced for five years, Vorhaus [54] attempted to evaluate the use of thiamine hydrochloride in the treatment of polyneuritis. He reported that the shorter the duration of symptoms, the quicker the response in most instances. Approximately 75 per cent of his patients revealed some improvement within four weeks.

It has been suggested recently that delirium tremens is essentially a condition of deficiency of thiamine. Investigators [55] observed that when thiamine hydrochloride was given intravenously, even in the presence of continued drinking, symptoms of delirium tremens disappeared. In cases of Korsakoff's psychosis, however, improvement was not noted after administration of thiamine hydrochloride.[56] In 20 patients who had severe, moderately severe and mild forms of Sydenham's chorea, marked improvement was noted after administration of the vitamin B complex.[57] It was noted also in animals [58] that the abstinence symptoms of morphine addiction were markedly decreased after administration of thiamine hydrochloride. Similar reports on clinical material have not yet appeared.

Bakhsh [59] reported the cases of 7 patients with trigeminal neuralgia to whom thiamine hydrochloride was given in doses of 10 to 30 mg.

53. Aring, C. D., and Spies, T. D.: A Critical Review: Vitamin B Deficiency and Nervous Disease, J. Neurol. & Psychiat. **2**:335-360 (Oct.) 1939.

54. Vorhaus, M. G.: Evaluation of Vitamin B_1 (Thiamin Chloride) in the Treatment of Polyneuritis, Am. J. M. Sc. **198**:837-844 (Dec.) 1939.

55. Kiene, H. E.; Streitwieser, R. J., and Miller, H.: The Role of Vitamin B_1 in Delirium Tremens, J. A. M. A. **114**:2191-2194 (June 1) 1940.

56. Bowman, K. M.; Goodhart, R., and Jolliffe, N.: Observations on the Role of Vitamin B_1 in the Etiology and Treatment of Korsakoff Psychosis, J. Nerv. & Ment. Dis. **90**:569-575 (Nov.) 1939.

57. Stone, S.: Treatment of Sydenham's Chorea by Fever and Vitamin B Therapy, New England J. Med. **223**:489-496 (Sept. 26) 1940.

58. Fitzhugh, O. G.: The Effect of Vitamin B_1 on Morphine Abstinence Symptoms, J. Pharmacol. & Exper. Therap. **67**:423-428 (Dec.) 1939.

59. Bakhsh, I.: Treatment of Nervous Diseases by Vitamin B_1 with Special Reference to Trigeminal Neuralgia: A Report of Seven Cases, Indian M. Gaz. **74**:456-458 (Aug.) 1939.

daily. Four patients experienced complete relief, and 2, partial relief; 1 patient was unaffected. The 4 patients whose relief of pain was dramatic began to improve within forty-eight hours after administration of thiamine hydrochloride. Others [60] observed relief of pain in neuritis of the eighth cranial nerve after administration of thiamine hydrochloride.

Equivocal results have been reported after the administration of thiamine hydrochloride to patients who had functional digestive disturbances,[61] multiple sclerosis,[62] dysphagia [63] or acrodynia.[64]

After administration of thiamine hydrochloride in doses of 5 mg. three times a day, Ochsner and Smith [65] observed marked relief of pain among patients who had varicose ulcers. The authors reported that if the symptoms do not subside within three to four days the dose should be doubled. Owens and his associates [66] made a critical study of vitamin B therapy for patients who have diabetes mellitus. In contradistinction to several claims made during the past year in which vitamin therapy was said to be of some benefit to patients with diabetes, these investigators pointed out that the administration of large amounts of thiamine hydrochloride and riboflavin in cases of well controlled diabetes over many weeks did not reduce the insulin requirement or alter in any way the severity of the diabetic state. The authors concluded that in cases of well controlled diabetes, except in those of diabetes mellitus, use of any thiamine hydrochloride or riboflavin beyond the amounts provided by the ordinary diabetic diet was not required. These authors pointed out that perhaps severe vitamin deficiency does play a part in diabetic coma. Certainly, this phase needs further clinical investigation.

Bean and Spies [67] stressed that chronic diarrhea predisposes to the development of a deficiency syndrome by causing increased loss of, or

60. Brandenburg, K. C.: Relief of Neuritis of Eighth Cranial Nerve with Vitamin B_1, Arch. Otolaryng. **31**:189-191 (Jan.) 1940.

61. Chesley, F. F.; Dunbar, J., and Crandall, L. A., Jr.: The Vitamin B Complex and Its Constituents in Functional Digestive Disturbances, Am. J. Digest. Dis. **7**:24-27 (Jan.) 1940.

62. Moore, M. T.: Treatment of Multiple Sclerosis with Nicotinic Acid and Vitamin B_1: Preliminary Report, Arch. Int. Med. **65**:1-20 (Jan.) 1940.

63. Jankelson, I. R.: Dysphagia Ascribed to Vitamin B Deficiency, Am. J. Digest. Dis. **7**:252-253 (June) 1940.

64. Durand, J. I.; Spickard, V. W., and Burgess, E.: Acrodynia Treated with Intramuscular Injections of Vitamin B_1, J. Pediat. **14**:74-78 (Jan.) 1939. Williams, P.; Shapiro, B. G., and Bartelot, R.: Treatment of Acrodynia with Vitamin B_1 Given Parenterally, Lancet **1**:76 (Jan. 13) 1940.

65. Ochsner, A., and Smith, M. C.: The Use of Vitamin B_1 for the Relief of Pain in Varicose Ulcers, J. A. M. A. **114**:947-948 (March 16) 1940.

66. Owens, L. B.; Rockwern, S. S., and Brown, E. G.: Evaluation of Vitamin B Therapy for Diabetes, Arch. Int. Med. **66**:679-687 (Sept.) 1940.

67. Bean, W. B., and Spies, T. D.: Vitamin Deficiencies in Diarrheal States, J. A. M. A. **115**:1078-1081 (Sept. 28) 1940.

decreased absorption of, vitamins. In addition to other vitamin supplements, these authors also suggested that 10 to 15 mg. of thiamine hydrochloride be given parenterally each day to patients who have diarrhea. Field and his associates [68] pointed out that patients who received intensive alkaline therapy for peptic ulcer and achlorhydria had subnormal urinary excretions of thiamine. Therefore, more thiamine hydrochloride should be administered to patients with such conditions than to a normal person if a deficiency is to be prevented.

In a study of beriberi, Weiss [69] could not establish any significant difference between oriental and occidental beriberi. He stated that the cardiovascular manifestations of beriberi are related definitely to a deficiency of thiamine. He pointed out:

> The evidence now available on the causative relation between thiamin deficiency and cardiovascular dysfunction of beriberi is (*a*) thiamin deficiency in the diet, (*b*) decreased thiamin content in the urine, (*c*) disappearance of the disease after the administration of thiamin chloride, (*d*) induction of electrocardiographic changes in healthy men consuming a diet lacking only thiamin chloride, (*e*) induction in animals on a thiamin deficient diet of cardiac disturbances, including cardiac dilatation, electrocardiographic changes, congestive failure of the circulation and structural changes similar to or identical with those observed in man, and (*f*) disappearance of the experimentally induced cardiovascular dysfunction in animals after administration of thiamin chloride.

NICOTINIC ACID

Chemical and Physiologic Properties.—As the authors of last year's review observed,[70] nicotinic acid bears a close relation to an enzyme system. Experimental studies seem to establish conclusively this relationship between nicotinic acid and the factor V (coenzymes 1 and 2 and possibly unknown related substances).[71] Although the content of these compounds in the blood is not uniformly diminished in cases of pellagra in human beings (and hence is not of diagnostic or prognostic value), the administration of nicotinic acid has been shown to increase almost

68. Field, H., Jr.; Robinson, W. D., and Melnick, D.: The Destruction of Thiamin by Unacidified Bile and Pancreatic Juice: A Possible Explanation of the Cord Changes in Pernicious Anemia, J. Clin. Investigation **19**:791 (Sept.) 1940.

69. Weiss, S.: Occidental Beriberi with Cardiovascular Manifestations: Its Relation to Thiamin Deficiency, J. A. M. A. **115**:832-839 (Sept. 7) 1940.

70. Wilder, R. M.; Browne, H. C., and Butt, H. R.: Diseases of Metabolism and Nutrition: Review of Certain Recent Contributions, Arch. Int. Med. **65**:390-460 (Feb.) 1940.

71. Axelrod, A. E., and Elvehjem, C. A.: Effect of Nicotinic Acid Deficiency on the Cozymase Content of Tissues, Nature, London **143**:281-282 (Feb. 18) 1939. Kohn, H. I.; Klein, J. R., and Dann, W. J.: The V-Factor Content and Oxygen Consumption of Tissues of the Normal and Blacktongue Dog, Biochem. J. **33**:1432-1442 (Sept.) 1939.

invariably the level of this factor in the blood of both normal persons [72] and patients who have pellagra. Although it is not known whether human organs undergo changes in the content of cozymase parallel with those observed in animal tissues, Elvehjem [73] stated that the rapid improvement produced in human beings and animals may be due to the rapid formation of cozymase. Kodicek [74] found little or no free nicotinic acid in living animal tissues and concluded that in autolysis of tissue this substance was set free from the coenzymes or from related compounds.

In contradiction to the previous finding of Vilter, Vilter and Spies, it was shown by Kohn and Klein [75] that erythrocytes of human blood can synthesize factor V from nicotinic acid in vitro.

Clinical investigations were carried out which demonstrated the role of nicotinic acid in carbohydrate metabolism with relation to diabetes,[76] in the administration of insulin [77] and in cerebral carbohydrate metabolism.[78] The last factor was suggested as the possible basis for an explanation of the mental changes observed in persons with pellagra. Sydenstricker and his associates [76] observed 2 patients with complicated diabetes in whom the clinical signs of pellagra appeared if the carbo-

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72. (*a*) Kohn, H. I.: The Concentration of Coenzyme-Like Substance in Blood Following Administration of Nicotinic Acid to Normal Individuals and Pellagrins, Biochem. J. **32**:2075-2083, 1938. (*b*) Vilter, R. W.; Vilter, S. P., and Spies, T. D.: Relationship Between Nicotinic Acid and a Codehydrogenase (Cozymase), J. A. M. A. **112**:420-422 (Feb. 4) 1939. (*c*) Kohn, H. I.; Bernheim, F., and Felsovanyi, A. V.: The Blood V-Factor (Coenzyme) Level in Normal and Pathological Subjects, J. Clin. Investigation **18**:585-591 (Sept.) 1939. (*d*) Axelrod, A. E.; Gordon, E. S., and Elvehjem, C. A.: The Relationship of the Dietary Intake of Nicotinic Acid to the Coenzyme I Content of Blood, Am. J. M. Sc. **199**:697-705 (May) 1940. (*e*) Axelrod, A. E.; Madden, R. J., and Elvehjem, C. A.: The Effect of Nicotinic Acid Deficiency upon the Coenzyme I Content of Animal Tissues, J. Biol. Chem. **131**:85-93 (Nov.) 1939. (*f*) Vilter, S. P.; Koch, M. B., and Spies, T. D.: Coenzymes I and II in Human Blood, J. Lab. & Clin. Med. **26**:31-44 (Oct.) 1940.

73. Elvehjem, C. A.: Relation of Nicotinic Acid to Pellagra, Physiol. Rev. **20**:249-271 (April) 1940.

74. Kodicek, E.: Estimation of Nicotinic Acid in Animal Tissues, Blood and Certain Foodstuffs: II. Applications, Biochem. J. **34**:724-735 (May) 1940.

75. Kohn, H. I., and Klein, J. R.: Synthesis of Factor V (Pyridine Nucleotides) from Nicotinic Acid in Vitro by Human Erythrocytes, J. Biol. Chem. **135**: 685-689 (Sept.) 1940.

76. Sydenstricker, V. P.; Geeslin, L. E., and Weaver, J. W.: Avitaminosis Occurring in Diabetic Patients Under Insulin Therapy, J. A. M. A. **113**:2137-2138 (Dec. 9) 1939.

77. Burke, J. C., and McIntyre, A. R.: Nicotinic Acid and the Vitamin B Complex in Insulin Tolerance, J. Pharmacol. & Exper. Therap. **67**:142-146 (Oct.) 1939.

78. Himwich, H. E.; Spies, T. D.; Fazekas, J. F., and Nesin, S.: Cerebral Carbohydrate Metabolism During Deficiency of Various Members of the Vitamin B Complex, Am. J. M. Sc. **199**:849-853 (June) 1940.

hydrate in the diet and the dose of insulin were increased. These symptoms could be controlled with nicotinic acid, but recurred whenever the intake of carbohydrates and insulin was increased again. They noted similar occurrences in a case of diabetes and combined deficiency of nicotinic acid and riboflavin. They concluded from these observations that, since "co-enzymes I and II both contain nicotinic acid, . . . it is quite possible that the great increase in dextrose metabolism . . . might exhaust the body stores of this vitamin." This is in keeping with the finding by Vilter and associates [72f] of a depleted coenzyme content in the blood of patients with diabetes who had ketosis. Sydenstricker and associates [76] further pointed out that the familiar glossitis and stomatitis occurring in persons with diabetes probably represent a vitamin deficiency rather than "lowered resistance" or a "toxic state."

Various methods have been suggested in the past for the chemical estimation of nicotinic acid, but the most satisfactory seems to be that which depends on the reaction with cyanogen bromide and aniline, which produces a yellow color. Numerous modifications of this procedure have been described during the past year.[79] Although it had previously been reported [80] that normal persons excrete between 20 and 50 mg. of nicotinic acid daily, more recent work has indicated that this value is probably too high and that the normal amount is between 3 and 12 mg. daily. Only traces of nicotinic acid have been recovered from the urine

79. (*a*) Swaminathan, M.: The Urinary Excretion of Nicotinic Acid, Indian J. M. Research **27**:417-428 (Oct.) 1939. (*b*) Bandier, E.: Quantitative Estimation of Nicotinic Acid in Urine, Biochem. J. **33**:1787-1793 (Nov.) 1939. (*c*) Harris, L. J., and Raymond, W. D.: Assessment of the Level of Nutrition: A Method for the Estimation of Nicotinic Acid in Urine, ibid. **33**:2037-2051 (Dec.) 1939. (*d*) Melnick, D., and Field, H., Jr.: Determination of Nicotinic Acid in Biological Materials by Means of Photoelectric Colorimetry, J. Biol. Chem. **134**:1-16 (June) 1940. (*e*) Schindel, L.: A Contribution to the Question of the Determination of Nicotinic Acid in Urine, J. Lab. & Clin. Med. **25**:515-516 (Feb.) 1940. (*f*) Melnick, D., and Field, H., Jr.: Chemical Determination of Nicotinic Acid: Inhibitory Effect of Cyanogen Bromide upon the Aniline Side Reactions, J. Biol. Chem. **135**: 53-58 (Aug.) 1940. (*g*) Najjar, V. A., and Wood, R. W.: Presence of a Hitherto Unrecognized Nicotinic Acid Derivative in Human Urine, Proc. Soc. Exper. Biol. & Med. **44**:386-390 (June) 1940. (*h*) Rosenblum, L. A., and Jolliffe, N.: Porphyrinuria in Pellagra, Am. J. M. Sc. **199**:853-858 (June) 1940; (*i*) Application to Urine of Bandier and Hald's Method for Determination of Nicotinic Acid, J. Biol. Chem. **134**:137-141 (June) 1940. (*j*) Melnick, D.; Robinson, W. D., and Field, H., Jr.: Influence of the Excretion of Other Pyridine Compounds upon the Interpretation of the Urinary Nicotinic Acid Values, ibid. **136**:131-144 (Oct.) 1940; (*k*) Urinary Excretion of Nicotinic Acid and Its Derivatives by Normal Individuals, ibid. **136**:145-156 (Oct.) 1940; (*l*) Factors Affecting the Concentration and Distribution of Nicotinic Acid in the Blood, ibid. **136**:157-166 (Oct.) 1940.

80. Vilter, S. P.; Spies, T. D., and Mathews, A. P.: A Method for the Determination of Nicotinic Acid, Nicotinamide, and Possibly Other Pyridine-Like Substances in Human Urine, J. Biol. Chem. **125**:85-98 (Sept.) 1938.

in the presence of a deficiency. Najjar and Wood [79g] discovered a substance in human urine which they were unable to identify but which was increased by administration of nicotinic acid. The presence of porphyrins in the urine was demonstrated to be of no value in the diagnosis of pellagra, but it probably reflects the presence of hepatic dysfunction.[79h] Methods of biologic assay include the effect on the growth of bacteria and the growth response of dogs with blacktongue.[81]

Since the discovery of the antipellagra properties of nicotinic acid, various related pyridine derivatives have been subjected to investigation for evidence of similar properties. From the work of Woolley and his associates [82] on dogs with blacktongue, it appeared that a rather specific structure was required to produce this activity. They concluded that substances depend for their antiblacktongue activity on their ability to undergo oxidative or hydrolytic conversion to nicotinic acid or its amide in the body.

In the activity of those compounds tried for the treatment of pellagra in human beings, there has been rather close correlation with the material in table 1 of Elvehjem.[73] The sodium and the ammonium salts of nicotinic acid were shown to possess activity [83] and the diethyl amide (coramine) demonstrated some activity.[84] Pyrazine-2.3-dicarboxylic acid, pyrazine monocarboxylic acid, thiazolecarboxylic acid and quinolinic acid exhibited limited activity in the treatment both of the pellagra of human beings and of the blacktongue of dogs. Spies and his associates [85] reported that patients who had pellagra and were in a state of relapse were benefited by the administration of adenylic acid, but its use was not recommended because of the severe reactions which were produced.

Clinical Use.—Some doubt still exists as to whether nicotinic acid is the true pellagra-preventive factor. If the rather pertinent suggestion

81. Elvehjem, C. A.; Waisman, H. A., and Axelrod, A. E.: Nicotinic Acid: Its Distribution, Function and Relation to the Other Members of the B Complex, J. Nutrition (supp.) **17**:11, 1939.

82. Woolley, D. W.; Strong, F. M.; Madden, R. J., and Elvehjem, C. A.: Anti-Black Tongue Activity of Various Pyridine Derivatives, J. Biol. Chem. **124**: 715-723 (Aug.) 1938.

83. Spies, T. D.; Bean, W. B., and Stone, R. E.: The Treatment of Subclinical and Classic Pellagra: Use of Nicotinic Acid, Nicotinic Acid Amide and Sodium Nicotinate, with Special Reference to the Vasodilator Action and the Effect on Mental Symptoms, J. A. M. A. **111**:584-590 (Aug. 13) 1938.

84. Sydenstricker, V. P.; Schmidt, H. L., Jr.; Fulton, M. C.; New, J. S., and Geeslin, L. E.: Treatment of Pellagra with Nicotinic Acid: Observations in Forty-Five Cases, South. M. J. **31**:1155-1163 (Nov.) 1938. Weinberg, M. H.: Case of Subclinical Pellagra—Psychotic Type—Treated Successfully with Coramine (Nicotinic Acid), Am. J. Psychiat. **96**:701-704 (Nov.) 1939.

85. Bills, C. E.; McDonald, F. G., and Spies, T. D.: Antipellagric Action of Pyrazine-2, 3-Dicarboxylic Acid and Pyrazine Monocarboxylic Acid, South. M. J. **32**:793-795 (Aug.) 1939. Spies, T. D.; Bean, W. B., and Vilter, R. W.: Adenylic Acid in Human Nutrition, Ann. Int. Med. **13**:1616-1618 (March) 1940.

of Sebrell and Butler [86] is followed and the diagnosis of pellagra is reserved for that syndrome which responds to the administration of nicotinic acid, this is then by definition the true pellagra-preventive factor. On the other hand, pellagra as encountered clinically is a disease of multiple deficiencies and must be treated as such. Taylor [87] made the interesting, though not new, suggestion that the cause of pellagra may be fourfold: (1) deficiency of the pellagra-preventive factor in the diet; (2) intrinsic deficiency in the gastric juice; (3) exposure to sunlight, and (4) an unidentified micro-organism. It should be remembered further that nicotinic acid alone will not prevent the recurrence of the cardinal symptoms of pellagra.[88] The introduction of vitamin B_6 and pantothenic acid may prove to be of great importance in the treatment and control of pellagra.

Jolliffe and his associates [89] reported on the observation of 150 cases of a syndrome characterized by clouding of the consciousness, cogwheel rigidities and uncontrollable grasping and sucking reflexes, either with or without manifestations of any deficiency disease. By treatment with nicotinic acid and hydration, they were able to reduce the mortality rate of this syndrome from 89.4, 100 or 51.5 per cent to 13.6 per cent. They suggested that this syndrome represented an acute, complete lack of nicotinic acid, in contrast to pellagra, which they regarded as a chronic deficiency.

The well known effect of nicotinic acid in producing peripheral vasodilalation has led to an investigation of the possibility of its use in the treatment of peripheral vascular disease. In a study of 15 normal adults, Abramson and his associates [90] found that after oral administration of nicotinic acid the flow of blood to the forearm and hand was increased as much as two and a half times and the effect lasted as long as one hundred and sixty minutes; only a slight increase was observed in the flow to the lower extremities. Appreciable changes in blood pressure, pulse rate or cutaneous temperatures were not noted. They did not find significant differences in these effects when the intravenous

86. Sebrell, W. H., Jr., and Butler, R. E.: Riboflavin Deficiency in Man (Ariboflavinosis), Pub. Health Rep. **54**:2121-2131 (Dec. 1) 1939.

87. Taylor, F. R.: The Etiology of Pellagra, Am. J. Digest. Dis. **6**:123-125 (April) 1939.

88. Schmidt, H. L., Jr., and Sydenstricker, V. P.: Nicotinic Acid in the Prevention of Pellagra: Preliminary Note, J. A. M. A. **110**:2065-2066 (June 18) 1938.

89. Jolliffe, N.; Bowman, K. M.; Rosenblum, L. A., and Fein, H. P.: Nicotinic Acid Deficiency in Encephalopathy, J. A. M. A. **114**:307-312 (Jan. 27) 1940. Jolliffe, N.: Effects of Vitamin Deficiency on Mental and Emotional Processes, A. Research Nerv. & Ment. Dis., Proc. (1938) **19**:114-153, 1939.

90. Abramson, D. I.; Katzenstein, K. H., and Senior, F. A.: Effect of Nicotinic Acid on Peripheral Blood Flow in Man, Am. J. M. Sc. **200**:96-102 (July) 1940.

rather than the oral route of administration was used. Estimations of cutaneous temperatures by Bean and Spies [91] revealed that only those compounds containing the radical produced vasodilatation and that related pellagra-preventive substances not containing this radical did not have a similar effect.[92] They discovered that the administration of aminoacetic acid will prevent the vasodilatation, probably through conversion of nicotinic acid to nicotinuric acid. Others [93] observed the failure of nicotinic acid amide to cause flushing of patients. Although they observed an increase in cutaneous temperatures up to 1.5 degrees C. (2.7 degrees F.), Bean and Spies [91] concluded that nicotinic acid will not prove to be of much value in the treatment of peripheral vascular disorders; indeed, they warned that its use may be dangerous.

Other conditions for which treatment with nicotinic acid has been suggested include otosclerosis,[94] Vincent's disease,[95] acne vulgaris,[96] infected scabies,[97] cyanosis resulting from the use of sulfanilamide [98] and xerostomia.[99] Frostig and Spies [92] reported the successful treatment of a type of psychoneurosis which they considered as resulting from a specific deficiency of nicotinic acid.

Calder and Kerby [100] observed that the administration of nicotinic acid in vitro to patients who had brucellosis reduced the clotting time of the blood to normal and effected normal retraction of the clot. They

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91. Bean, W. B., and Spies, T. D.: A Study of the Effects of Nicotinic Acid and Related Pyridine and Pyrazine Compounds on the Temperature of the Skin of Human Beings, Am. Heart J. **20**:62-76 (July) 1940.

92. Frostig, J. P., and Spies, T. D.: The Initial Nervous Syndrome of Pellagra and Associated Deficiency Diseases, Am. J. M. Sc. **199**:268-274 (Feb.) 1940.

93. Field, H. J., and Robinson, W. D.: The Absence of Reactions Following Therapeutic Doses of Nicotinic Acid Amide, Am. J. M. Sc. **199**:275-276 (Feb.) 1940.

94. Selfridge, G.: Nicotinic Acid in the Eighth Nerve: Preliminary Report, Ann. Otol., Rhin. & Laryng. **48**:39-53 (March) 1939; The Eighth Nerve in Relation to Thiamin Chloride and Nicotinic Acid: A Comparative Study, ibid. **48**:419-432 (June) 1939.

95. King, J. D.: Vincent's Disease Treated with Nicotinic Acid, Lancet **2**:32-35 (July 13) 1940.

96. Lynch, F. W.: Nicotinic Acid in the Treatment of Acne Vulgaris, Arch. Dermat. & Syph. **42**:481-482 (Sept.) 1940.

97. Goodall, J. W. D.: Observations on the Use of Nicotinic Acid in the Treatment of Pellagra and Allied Conditions, Indian M. Gaz. **75**:147-153 (March) 1940.

98. Doughty, J. F.: Sulfanilamide Cyanosis Relieved by Nicotinic Acid, J. A. M. A. **114**:756 (March 2) 1940.

99. Saphir, W.: Xerostomia Successfully Treated with Nicotinic Acid, Am. J. Digest. Dis. **7**:298-299 (July) 1940.

100. Calder, R. M., and Kerby, G. P.: The Effect of Nicotinic Acid on Blood Coagulation, Am. J. M. Sc. **200**:590-596 (Nov.) 1940.

reported that administration of nicotinic acid to patients who had hemorrhagic tendencies (typhoid fever, hypoplastic anemia or acute catarrhal jaundice) seemed to bring about correction of this disorder. Effects were not noted when the blood of patients who had hemophilia was used. An explanation of the mechanism by which these changes may be produced was not offered.

The proper dose of nicotinic acid has not yet been properly determined. Apparently, the drug is relatively nontoxic and the unpleasant flushing effect would seem to be without harm. Nicotinic acid amide does not produce this flushing.[101]

RIBOFLAVIN

Chemical and Physiologic Properties.—Studies of distribution of riboflavin reveal that the liver and kidneys of swine, cattle, lambs and calves contain comparatively large amounts of riboflavin and that the muscular tissues of these animals contain less riboflavin than do the glandular tissues. Riboflavin was not lost from these tissues by the ordinary household method of stewing, but an appreciable amount was lost when samples were roasted or fried.[102] It has been known for several years that tubercle bacilli contain a yellow pigment (phthiocol 1 [2-methyl-3-hydroxy-1, 4-naphthoquinone]), which recently has been shown to possess marked antihemorrhagic activity, similar to that of vitamin K. Street and Reeves[103] demonstrated more recently that a fluorescent yellow pigment in the tubercle bacillus apparently is identical with riboflavin.

During the last year several methods for determining the riboflavin content of foodstuffs[104] and the urinary excretion of riboflavin[105] were reported, but it is believed by most investigators that these methods can be improved.

Clinical Deficiency.—That a deficiency of riboflavin occurs among human beings was reported by Sebrell and Butler,[106] as cited in this

101. Frostig and Spies.[92] Saphir.[99]

102. Mickelsen, O.; Waisman, H. A., and Elvehjem, C. A.: The Distribution of Riboflavin in Meat and Meat Products, J. Nutrition **18**:517-526 (Nov.) 1939.

103. Street, H. R., and Reeves, R. E.: Occurrence of Riboflavin in Tubercle Bacillus, Proc. Soc. Exper. Biol. & Med. **44**:641-644 (June) 1940.

104. Hodson, A. Z., and Norris, L. C.: A Fluorometric Method for Determining the Riboflavin Content of Foodstuffs, J. Biol. Chem. **131**:621-630 (Dec.) 1939.

105. Ferrebee, J. W.: The Urinary Excretion of Riboflavin: Fluorometric Methods for Its Estimation, J. Clin. Investigation **19**:251-256 (Jan.) 1940. Fraser, H. F.; Topping, N. H., and Isbell, H.: The Bacterial Assay of Riboflavin in the Urine and Tissues of Normal and Depleted Dogs and Rats, Pub. Health Rep. **55**: 280-289 (Feb. 16) 1940.

106. Sebrell, W. H., and Butler, R. E.: Riboflavin Deficiency in Man: A Preliminary Note, Pub. Health Rep. **53**:2282-2284 (Dec. 30) 1938.

review last year. This original work of Sebrell and Butler has now been amply confirmed by several investigators,[107] and within the past year Sebrell and his co-workers [108] have reported other symptoms which often are present among persons who subsist on a diet deficient in riboflavin. In addition to cheilosis and seborrheic accumulations at the nasolabial fold, they described a specific type of glossitis which often can be recognized before other signs of deficiency of riboflavin are present. The tongue is clean; the papillae are flat rather than atrophic, and the color is definitely purplish red or magenta, as compared with the scarlet-red tongue so often seen in patients with deficiency of nicotinic acid. These investigators pointed out that this type of glossitis often develops in patients with pellagra after the red and atrophic tongue has become normal in appearance from treatment with nicotinic acid while the diet has remained deficient in riboflavin. In addition to this newly observed characteristic of deficiency of riboflavin, the authors also described certain ocular changes which nearly always are present in such a deficiency. These ocular changes can be seen easily with a slit lamp. The earliest lesion is proliferation and engorgement of the limbic plexus, which progresses to superficial vascularization of the cornea and the production of interstitial keratitis. Photophobia, congestion of the sclera, vascularization and abnormal pigmentation of the iris, dimness of vision and actual impairment of visual acuity are relieved promptly, and at times dramatically, by the administration of riboflavin. From these studies the authors suggested that deficiency of riboflavin is perhaps one of the most prevalent forms of uncompensated avitaminosis. Similar results were obtained by Johnson and Eckardt.[109]

It has also been reported [110] that riboflavin is of value in the treatment of pemphigus.

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107. Sydenstricker, V. P.; Geeslin, L. E.; Templeton, C. M., and Weaver, J. W.: Riboflavin Deficiency in Human Subjects, J. A. M. A. **113**:1697-1700 (Nov. 4) 1939. Jolliffe, N.; Fein, H. D., and Rosenblum, L. A.: Riboflavin Deficiency in Man, New England J. Med. **221**:921-926 (Dec. 14) 1939. Shields, W. P.: Riboflavin Deficiency: Report of a Case in a Child with Cure by Specific Treatment, ibid. **223**:215-216 (Aug. 8) 1940.

108. Kruse, H. D.; Sydenstricker, V. P.; Sebrell, W. H., and Cleckley, H. M.: Ocular Manifestations of Riboflavinosis, Pub. Health Rep. **55**:157-169 (Jan. 26) 1940. Sydenstricker, V. P.; Sebrell, W. H.; Cleckley, H. M., and Kruse, H. D.: The Ocular Manifestations of Ariboflavinosis: A Progress Note, J. A. M. A. **114**: 2437-2445 (June 22) 1940.

109. Johnson, L. V., and Eckardt, R. E.: Rosacea Keratitis and Conditions with Vascularization of Cornea Treated with Riboflavin, Arch. Ophth. **23**:899-907 (May) 1940.

110. Topping, M. C., and Knoefel, A. F.: Use of Vitamin G in Pemphigus: Report of a Case of Pemphigus Vulgaris Treated Successfully with Riboflavin, J. A. M. A. **114**:2102 (May 25) 1940.

PYRIDOXINE (VITAMIN B_6)

Chemical and Physiologic Properties.—It has been suggested [111] and accepted by many writers that pyridoxine be adopted as the chemical name for vitamin B_6 (2-methyl-3-hydroxy-4, 5-di-hydroxymethyl-pyridine). Like other members of the vitamin B complex, pyridoxine (administered both as the base and as the hydrochloride) was found experimentally to be relatively free from toxic action.[112] Excessively large doses (3 Gm. per kilogram of body weight) produced convulsions and death in certain experimental animals, but long feeding of sublethal doses failed to produce symptoms of toxicity, which suggested that pyridoxine is either rapidly excreted or destroyed. Weigand and associates [113] did not observe any ill effects on man after the oral or intravenous administration of pyridoxine hydrochloride in doses of 100 to 200 mg.

Colorimetric methods for measuring the urinary excretion of pyridoxine (vitamin B_6) were described.[114] An average of 8.7 per cent of 50 mg. of pyridoxine given intravenously was recovered within an hour from the urine of a group of healthy human beings, and 7.6 per cent of 100 mg. administered orally was recovered from the urine within four hours.

Clinical Deficiency.—It was reported last year [115] that a syndrome characterized by "extreme nervousness, insomnia, irritability, abdominal pain, weakness and difficulty in walking" dramatically disappeared after intravenous administration of pyridoxine. This report was not confirmed by Kark and his associates,[116] but since that time Spies and his co-workers [117] have treated 20 patients with similar conditions and have obtained good results. In a further study of this problem, these authors

111. György, P., and Eckardt, R. E.: Vitamin B_6 and Skin Lesions in Rats, Nature, London **144**:512 (Sept. 16) 1939.

112. Unna, K., and Antopol, W.: Toxicity of Vitamin B_6, Proc. Soc. Exper. Biol. & Med. **43**:116-118 (Jan.) 1940.

113. Weigand, C. G.; Eckler, C. R., and Chen, K. K.: Action and Toxicity of Vitamin B_6 Hydrochloride, Proc. Soc. Exper. Biol. & Med. **44**:147-151 (May) 1940.

114. Scudi, J. V.; Koones, H. F., and Keresztesy, J. C.: Urinary Excretion of Vitamin B_6 in the Rat, Proc. Soc. Exper. Biol. & Med. **43**:118-122 (Jan.) 1940. Scudi, J. V.; Unna, K., and Antopol, W.: A Study of the Urinary Excretion of Vitamin B_6 by a Colorimetric Method, J. Biol. Chem. **135**:371-376 (Sept.) 1940.

115. Spies, T. D.; Bean, W. B., and Ashe, W. F.: A Note on the Use of Vitamin B_6 in Human Nutrition, J. A. M. A. **112**:2414-2415 (June 10) 1939.

116. Kark, R.; Lozner, E. L., and Meiklejohn, A. P.: Negative Effect of Synthetic Vitamin B_6 Hydrochloride in Nutritional Deficiency in Man, Proc. Soc. Exper. Biol. & Med. **43**:97-99 (Jan.) 1940.

117. Spies, T. D.; Ladisch, R. K., and Bean, W. B.: Vitamin B_6 (Pyridoxin) Deficiency in Human Beings: Further Studies, with Special Emphasis on the Urinary Excretion of Pyridoxin, J. A. M. A. **115**:839-840 (Sept. 7) 1940.

observed that within an hour after intravenous injection of pyridoxine the average urinary excretion of this material by normal persons is 7.9 per cent and by patients with pellagra 8.6 per cent. Four patients of the group studied were suspected of having a deficiency of pyridoxine, and the lowest rate of excretion (averaging 0.2 per cent) of pyridoxine occurred among them. The absorption of pyridoxine apparently is dependent on the diet, as patients with a clinical deficiency disease who are placed on a diet deficient in pyridoxine are found to excrete none of the injected material. It was concluded from these results that pyridoxine is important to human nutrition, and this particular investigation gave strong support to the hypothesis, repeatedly mentioned by Spies, that a clinical deficiency disease occurs not as a single entity but as a complex syndrome.

Cheilosis is considered to be the result of a deficiency of riboflavin, but Smith and Martin [118] reported that cheilosis can be treated successfully with synthetic pyridoxine hydrochloride. The authors reported several cases of typical cheilosis resulting from a deficiency of riboflavin in which a rather dramatic response occurred after administration of pyridoxine hydrochloride. The patient in 1 case which the authors reported had mild cheilosis associated with sprue. Although slight improvement of the lesions of the mouth occurred after administration of pyridoxine hydrochloride, complete healing did not result either from pyridoxine hydrochloride in doses of 1,000 mg. or from a combination of pyridoxine hydrochloride (300 mg.), riboflavin (140 mg.) and nicotinic acid (1,000 mg.). Finally the lesion healed after administration of 445 U. S. P. units of a concentrated liver extract given for one week. The authors gave three possible explanations for this peculiar observation: 1. A primary and specific deficiency of riboflavin is responsible for cheilosis, and pyridoxine acts only indirectly. 2. A primary and specific deficiency of pyridoxine is responsible for cheilosis, and riboflavin acts only indirectly. 3. Both riboflavin and pyridoxine are necessary to maintain the integrity of the lip at the mucocutaneous junction, and a deficiency of either will precipitate the lesion.

It has been well established that hypochromic anemia, which develops among dogs on a diet deficient in pyridoxine, is not relieved by the administration of iron.[119] In this connection, Vilter and his associates [120]

118. Smith, S. G., and Martin, D. W.: Cheilosis Successfully Treated with Synthetic Vitamin B_6, Proc. Soc. Exper. Biol. & Med. **43**:660-663 (April) 1940.

119. McKibbin, J. M.; Madden, R. J.; Black, S., and Elvehjem, C. A.: The Importance of Vitamin B_6 and Factor W in the Nutrition of Dogs, Am. J. Physiol. **128**:102-110 (Dec.) 1939.

120. Vilter, R. W.; Schiro, H. S., and Spies, T. D.: Effect of Synthetic Vitamin B_6 on the Haemopoietic System of Human Beings, Nature, London **145**: 388 (March 9) 1940.

reported an interesting study on the effect of pyridoxine on the hemopoietic system of human beings. To 3 patients who had pellagra and macrocytic anemia and to 2 patients who had pernicious anemia, from 50 to 100 mg. of crystalline pyridoxine in sterile physiologic solution of sodium chloride was administered intravenously each day for ten days. The patients experienced a sense of well-being and increased strength within forty-eight hours, and on the fifth, sixth, seventh and eighth days a slight but definite reticulocytosis appeared in each instance. The reticulocyte count did not exceed 5 per cent, but the leukocyte count, which was low in the 2 cases of pernicious anemia, increased during this period. The increase was principally in the polymorphonuclear leukocytes. In another experiment, 100 mg. of pyridoxine was incubated with 100 cc. of gastric juice from the stomachs of normal fasting human beings. This material was administered orally to 1 of the patients who had pernicious anemia; a response identical with that observed after the intravenous administration of pyridoxine was obtained. These findings suggested that pyridoxine administered in large amounts has a definite effect on the hemopoietic system of patients who are in a state of relapse as a result of macrocytic anemia associated with pellagra or pernicious anemia.

One of the most interesting reports of the possible use of pyridoxine in the treatment of human diseases was that of Jolliffe [121] on its effect in cases of Parkinson's syndrome. This author selected 15 patients who had paralysis agitans and who were confined to bed or chair. To each of these patients 50 or 100 mg. of pyridoxine hydrochloride was administered intravenously either every day or every other day. Of these 15 patients, 4 revealed subjective and definite objective improvement and 2 additional patients were subjectively improved. The conditions of these patients represented so-called arteriosclerotic, or idiopathic, and postencephalitic types of paralysis agitans. The beneficial effects of the administration of this compound were limited to lessening of rigidity and to an increase in strength. Tremor was not affected.

Other beneficial effects of pyridoxine on diseases of the nervous system have been observed by Vilter and his associates.[122] These investigators reported that partial remission of a severe polyneuritic syndrome caused by arsenic occurred after administration of pyridoxine in 1 case. The most spectacular remission in this particular case occurred when pyridoxine and alpha tocopherol were administered.

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121. Jolliffe, N.: Clinical Aspects of Vitamin B Deficiencies, Minnesota Med. **23**:542-551 (Aug.) 1940.

122. Vilter, R. W.; Aring, C. D., and Spies, T. D.: A Case of Arsenic Peripheral Neuritis Treated with Synthetic Vitamin B_6 and Alpha-Tocopherol, J. A. M. A. **115**:209-211 (July 20) 1940.

PANTOTHENIC ACID

It has been known for some time that a factor curative for chicken dermatitis is found in filtered extracts of liver or yeast. This factor was called "filtrate factor" or "chicken antidermatitis factor." Several investigators have shown that the growth of the chicken was proportional to the quality of filtrate factor in the diet, and others have demonstrated that nicotinic acid and various other vitamins did not cure chicken dermatitis. While this work was being carried out, Williams and his associates,[123] working along different lines, determined that distributed apparently through all living tissue was a substance which they called "pantothenic acid." Soon these authors established that pantothenic acid consisted of "beta-alanine and a hydroxy acid." In 1939 Woolley and his associates[124] and Jukes,[125] working independently, announced that the chick antidermatitis factor (filtrate factor of Jukes[125]) appeared to be identical with the pantothenic acid described by Williams and his associates. Finally, in 1940, Williams and Major[126] announced the synthesis of pantothenic acid and described its formula. Pantothenic acid is a condensation product of beta alanine and alphahydroxybetabetadimethylgammabutyrolactone. This compound is destroyed by strong alkali and by dry heat, but it seems to be resistant to ordinary oxidizing and reducing agents.

Waisman and his associates[127] have reported that the liver and kidneys of various animals are the richest sources of pantothenic acid but that muscular tissues of cattle, lambs, swine and calves are poor sources. Ordinary stewing was found to decrease the potency of kidney, heart and spleen by a third, but the factor present in liver was not destroyed by frying. Jukes[128] found that extract of rice bran and bakers' yeast were excellent sources of this factor and that egg yolks, dried skimmed milk and alfalfa were also good sources. Carrots, onions

123. Rohrman, E.; Burget, G. E., and Williams, R. J.: Pantothenic Acid Content of Animal Tissues, Proc. Soc. Exper. Biol. & Med. **32**:473-474 (Dec.) 1934. Williams, R. J.; Mosher, W. A., and Rohrman, E.: The Importance of "Pantothenic Acid" in Fermentation, Respiration and Glycogen Storage, Biochem. J. **30**:2036-2039, 1936.

124. Woolley, D. W.; Waisman, H. A., and Elvehjem, C. A.: Studies on the Structure of the Chick Antidermatitis Factor, J. Biol. Chem. **129**:673-679 (Aug.) 1939.

125. Jukes, T. H.: Pantothenic Acid and the Filtrate (Chick Anti-Dermatitis) Factor, J. Am. Chem. Soc. **61**:975-976 (April) 1939.

126. Williams, R. J., and Major, R. T.: The Structure of Pantothenic Acid, Science **91**:246 (March 8) 1940.

127. Waisman, H. A.; Mickelsen, O., and Elvehjem, C. A.: The Distribution of the Chick Antidermatitis Factor (Pantothenic Acid) in Meats and Meat Products, J. Nutrition **18**:247-256 (Sept.) 1939.

128. Jukes, T. H.: Further Observations on the Assay, Distribution and Properties of the Filtrate Factor, J. Biol. Chem. **117**:11-20 (Jan.) 1937.

and canned green peas were poor sources. Rolled oats, whole corn meal, canned Alaska salmon and fresh beef were found to be fair sources.

A variety of interesting occurrences take place among animals on a diet deficient in pantothenic acid. In the chick a condition of dermatitis rapidly results which is characterized by lesions on the eyelids, at the corners of the mouth and on the legs and feet. Some workers[129] observed that the spinal cord of animals deficient in pantothenic acid is affected and concluded that pantothenic acid is necessary to keep the normal structure of the spinal cord intact. When rats subsisted on a diet deficient in this compound cutaneous lesions and changes involving depigmentation of the fur of black rats and "rusting" of albino rats developed.[130]

The only available reports on the effect of pantothenic acid and on its metabolism in human beings are those of Spies and his associates.[131] These authors observed that doses of 100 mg. of calcium or sodium pantothenate could be administered intravenously to human beings without any untoward reaction or changes in blood pressure, pulse rate, temperature or respiration. It was observed that the pantothenic acid content of the blood of 18 normal persons was between 1,900 and 3,200 micrograms per cubic centimeter and averaged 2,200 micrograms per cubic centimeter. It was observed further that after the intravenous injection of pantothenic acid, the content of the acid in the blood might increase to as much as 50 per cent above the preinjection level but that most of this increase was noted within the first three hours after injection. In each instance, however, the amount returned to its previous level within twenty-four hours. Analysis of specimens of urine likewise disclosed an increase in concentration of pantothenic acid after injection; the concentration returned to normal within twenty-four hours. In the blood of 28 patients who had pellagra, beriberi or a deficiency of riboflavin, the average level of pantothenic acid was only 5 to 900 micrograms per cubic centimeter. This was 23 to 50 per cent below the normal average.

After the intravenous injection of pantothenic acid, these authors also observed an increase of from 20 to 30 per cent in the level of riboflavin in the blood. Interestingly enough, the intravenous injection of 200 micrograms of riboflavin per kilogram of body weight was followed by an 80 per cent increase in the concentration of riboflavin in the blood

129. Phillips, P. H., and Engel, R. W.: Some Histopathologic Observations on Chicks Deficient in the Chick Antidermatitis Factor or Pantothenic Acid, J. Nutrition **18**:227-232 (Sept.) 1939.

130. György, P.; Poling, C. E., and Subbarow, Y.: Observations on the Factor Curative of Nutritional Achromatrichia, J. Biol. Chem. **132**:789-790 (Feb.) 1940.

131. Spies, T. D.; Stanbery, S. R.; Williams, R. J.; Jukes, T. H., and Babcock, S. H.: Pantothenic Acid in Human Nutrition, J. A. M. A. **115**:523-524 (Aug. 17) 1940. Stanbery, S. R.; Snell, E. E., and Spies, T. D.: Unpublished data.

and a 45 per cent increase in the level of pantothenic acid. These values remained elevated for three or four hours and in each instance returned to their former levels by the next day. These studies strongly indicate that perhaps pantothenic acid is essential to human nutrition and that its function is associated closely with that of riboflavin.

ASCORBIC ACID (VITAMIN C)

Chemical and Physiologic Properties.—The possibility of ascorbic acid functioning in an oxidation-reduction enzyme system has been recognized for several years,[132] but experimental proof of this action is still lacking. Investigations in this direction during the past year have failed to produce any concrete evidence that ascorbic acid acts as a respiratory enzyme or coenzyme. Schultze and his associates [133] demonstrated that ascorbic acid does not function as an agent in the transfer of hydrogen in a system containing nicotine hemochromogen, ascorbic acid, coenzyme, dextrose dehydrogenase and dextrose; further, cozymase did not reduce dehydroascorbic acid in vitro. Because of the circumstantial evidence for such a function of ascorbic acid, the problem remains an intriguing one and merits further investigation, as suggested in a recent editorial in *The Journal of the American Medical Association.*[134]

> It may well be that the efficacy of the vitamin as an antiscorbutic agent is related to a specific role in either oxidation-reduction reactions or to reactions that are responsible to a considerable degree for the transfer of hydrogen or electrons in the oxidation of metabolites.

The same editorial alluded to the work of Sealock and Silberstein,[135] who used guinea pigs that had alkaptonuria, and cited this work as "perhaps . . . an in vivo example of a relation of ascorbic acid to oxidation processes . . ." However, one of these investigators (Sealock) and other associates were unable to modify the excretion of homogentisic acid by the administration of 1 to 4 Gm. of ascorbic acid daily to a patient with alkaptonuria.[136]

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132. King, C. G.: Vitamin C, Ascorbic Acid, Physiol. Rev. **16**:238-262 (April) 1936.

133. Schultze, M. O.; Harrer, C. J., and King, C. G.: Studies on the Possible Carrier Rôle of Ascorbic Acid in Animal Tissues, J. Biol. Chem. **131**:5-12 (Nov.) 1939.

134. Possible Role of Ascorbic Acid in Animal Tissues, editorial, J. A. M. A. **114**:1668-1669 (April 27) 1940.

135. Sealock, R. R., and Silberstein, H. E.: The Excretion of Homogentisic Acid and Other Tyrosine Metabolites by the Vitamin C-Deficient Guinea Pig, J. Biol. Chem. **135**:251-258 (Aug.) 1940.

136. Sealock, R. R.; Galston, M., and Steele, J. M.: Administration of Ascorbic Acid to an Alkaptonuric Patient, Proc. Soc. Exper. Biol. & Med. **44**: 580-583 (June) 1940.

MacLean and associates [137] conducted an interesting experiment on guinea pigs that were on a diet deficient in ascorbic acid. They found that the principal lesion was related to the differentiation of the mesenchymal cells into osteoblasts and the stimulation of these osteoblasts to secrete bone matrix. They could not produce any evidence in support of the "jellation theory" of Wolbach and Howe. The formation of dentine in the teeth of guinea pigs attracted the attention of one group of investigators,[138] who found that in animals deficient in ascorbic acid the odontoblasts produced histologically normal dentine, but that the quantity produced was decreased in proportion to the inadequacy of vitamin C in the diet. They suggested the use of their procedures in the biologic assay of ascorbic acid.

The role of vitamin C in metabolism of calcium was studied by Lanford,[139] who observed that guinea pigs fed orange juice stored 8 per cent more calcium from the diet than did control animals. Others [140] were unable to note any effect of ascorbic acid on the serum calcium of guinea pigs, but Todhunter and Brewer [140a] found that the phosphatase activity of the serum was markedly decreased in cases of severe scurvy. They concluded that this diminution was the result of the scurvy and not of the restricted intake of food. It had been suggested previously that ascorbic acid was a powerful activator of serum phosphatase.[141] Chu and his associates,[142] in a comprehensive study of the metabolism of calcium and phosphorus in 2 cases of mild scurvy and 1 case of osteomalacia, found that the bodily retention and serum content of these elements were unaffected by the administration of ascorbic acid.

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137. MacLean, D. L.; Sheppard, M., and McHenry, E. W.: Tissue Changes in Ascorbic Acid Deficient Guinea-Pigs, Brit. J. Exper. Path. **20**:451-457 (Dec.) 1939.

138. Boyle, P. E.; Bessey, O. A., and Howe, P. R.: Rate of Dentin Formation in Incisor Teeth of Guinea Pigs on Normal and on Ascorbic Acid-Deficient Diets, Arch. Path. **30**:90-107 (July) 1940.

139. Lanford, C. S.: The Effect of Orange Juice on Calcium Assimilation, J. Biol. Chem. **130**:87-95 (Sept.) 1939.

140. (*a*) Todhunter, E. N., and Brewer, W.: The Ascorbic Acid, Phosphatase and Calcium Content of the Blood of Guinea Pigs with Varying Degrees of Scurvy, Am. J. Physiol. **130**:310-318 (Aug.) 1940. (*b*) Henry, K. M., and Kon, S. K.: The Retention of Calcium by the Rat in the Presence and in the Absence of Vitamin C, Biochem. J. **33**:1652-1654 (Oct.) 1939.

141. Maddock, S.; Thannhauser, S. J.; Reichel, M., and Grattan, J.: A New Conception of Serum Phosphatase: Review of Experimental Work, New England J. Med. **218**:166-169 (Jan. 27) 1938.

142. Chu, H. I.; Liu, S. H.; Ch'en, K. C.; Yu, T. F.; Hsu, H. C., and Cheng, T. Y.: The Effect of Vitamin C on the Calcium, Phosphorus and Nitrogen Metabolism in Scurvy and Osteomalacia, Chinese J. Physiol. **15**:101-118 (Jan. 30) 1940.

Shepherd and his associates [143] noted some effect of ascorbic acid on the retention of calcium, phosphorus and nitrogen in children.

That loss of resistance to infections was caused by deficiency of ascorbic acid has been postulated in the past; the experimental work has given rise to varying conclusions. During the last year, investigations of this problem in the field of animal experimentation have been rather meager. King and his associates [144] produced marked injury with diphtheria toxin to the odontoblasts and dentine in the teeth of guinea pigs and were able to prevent this by administration of ascorbic acid. Sigal [145] demonstrated the protective action of vitamin C against changes in the cells of circulating blood produced by diphtheria toxin. Torrance [146] found a significant reduction in the content of ascorbic acid in the skin of rabbits and guinea pigs at the site of injection of diphtheria toxin, but this reduction was no greater than that produced by thermal burns; he concluded that these changes were dependent on the inflammatory reaction and were not of a specific nature.

Murakami,[147] in a study of the hepatic function of guinea pigs deficient in ascorbic acid, demonstrated a reduction in the amount of bile secreted, in the pigment-excreting function (azorubin S test) and in the capacity to detoxify indole. All of these factors were restored to normal levels by administration of ascorbic acid.

Metabolism of Vitamin C.—Farmer, Abt and Chinn [148] demonstrated rather conclusively that the absorption of ascorbic acid from the intestinal tract of healthy persons is nearly complete, as the amount excreted in

143. Shepherd, M. L.; Macy, I.; Hunscher, H. A., and Hummel, F. C.: Synthesized, Processed, and Natural Sources of Vitamin C in the Mineral Metabolism of Normal Children, J. Pediat. **16**:704-716 (June) 1940.

144. King, C. G.; Musulin, R. R., and Swanson, W. F.: Effects of Vitamin C Intake upon the Degree of Tooth Injury Produced by Diphtheria Toxin, Am. J. Pub. Health **30**:1068-1072 (Sept.) 1940.

145. Sigal, A.: Effects of Vitamin C Deficiency and Diphtheria Toxin on Cellular Blood Constituents of Guinea Pig, Proc. Soc. Exper. Biol. & Med. **42**:163-167 (Oct.) 1939.

146. Torrance, C. C.: The Ascorbic Acid Concentration of Inflammatory Lesions of the Skin, J. Infect. Dis. **67**:53-58 (July-Aug.) 1940.

147. Murakami, O.: The Influence of Ascorbic Acid upon the Liver Function and the Mutual Relation Between Vitamins B_1 and C: I. The Influence of Ascorbic Acid upon the Pigment-Excreting Function of the Liver, Jap. J. Gastroenterol. **11**:1-6 (July) 1939; II. The Influence of Ascorbic Acid upon the Sodium Santonicum-Managing Function of the Liver, ibid. **11**:7-12 (July) 1939; III. The Influence of Ascorbic Acid upon the Indol-Detoxicating Function of the Liver, ibid. **11**:12-23 (July) 1939; IV. The Mutual Relationship Between Vitamins B_1 and C Regarding the Detoxicating Function of the Liver for Indol, ibid. **11**:24-30 (July) 1939.

148. Farmer, C. J.; Abt, A. F., and Chinn, H.: The Absorption of Vitamin C (*l*-Ascorbic Acid) from the Intestinal Tract, in Health and Disease, Quart. Bull., Northwestern Univ. M. School **14**:114-119, 1940.

the feces is usually less than 5 mg. in twenty-four hours. From experiments with guinea pigs, it appeared that this absorption occurred through a process of osmosis and diffusion and that phosphorylation was not required. Although they failed to find any increased intestinal excretion in the presence of high levels of ascorbic acid in the blood plasma, they regarded the possibility of reexcretion into the intestinal tract as unlikely. In the presence of increased intestinal motility, however, Abt, Farmer and Topper [149] found as much as 52 mg. of ascorbic acid in a twenty-four hour sample of feces, and they cautioned that the loss by such routes may be severe enough to deplete the ascorbic acid in the plasma. Wright and Ludden [150] found 380 mg. of ascorbic acid in the stools in twenty-four hours in cases of chronic diarrhea. They devised a formula for determining deficiency of absorption, which obviates the necessity of determining the ascorbic acid in the stools.

Sherry and his co-workers,[151] who performed clearance tests with ascorbic acid and creatinine on dogs, reaffirmed the opinion that ascorbic acid is excreted by glomerular filtration and active tubular reabsorption. This reabsorption mechanism appeared limited by a maximal rate, and if this rate was exceeded by the rate at which the vitamin was supplied by the glomerular filtrate the entire excess quantity was excreted in the urine. Although ascorbic acid is, in a general sense, a threshold substance, tubular reabsorption is never complete in human beings; if the level of ascorbic acid in the plasma is less than 1.5 mg. per hundred cubic centimeters the clearance of ascorbic acid is constant and independent of the concentration in the plasma.[152] Barbiturates and certain analgesic drugs,[153] the salicylate group in particular,[154] produce diuresis of ascorbic acid in animals. This observation is interpreted by some as evidence of increased endogenous formation of the vitamin in relation

149. Abt, A. F.; Farmer, C. J., and Topper, Y. J.: Influence of Catharsis and Diarrhea on Gastrointestinal Absorption of Ascorbic Acid in Infants, Proc. Soc. Exper. Biol. & Med. **43**:24-26 (Jan.) 1940.

150. Wright, I., and Ludden, J. B.: Treatment with Vitamin C (Cevitamic Acid-Ascorbic Acid): Method for Compensating for the Factor of Error Due to Renal Retention of Vitamin C Found in All Previous Saturation and Blood Tests, M. Clin. North America **24**:743-758 (May) 1940.

151. Sherry, S.; Friedman, G. J.; Paley, K.; Berkman, J., and Ralli, E. P.: The Mechanism of the Excretion of Vitamin C by the Dog Kidney, Am. J. Physiol. **130**:276-280 (Aug.) 1940.

152. Friedman, G. J.; Sherry, S., and Ralli, E. P.: The Mechanism of the Excretion of Vitamin C by the Human Kidney at Low and Normal Plasma Levels of Ascorbic Acid, J. Clin. Investigation **19**:685-689 (Sept.) 1940.

153. Longenecker, H. E.; Fricke, H. H., and King, C. G.: The Effect of Organic Compounds upon Vitamin C Synthesis in the Rat, J. Biol. Chem. **135**:497-510 (Sept.) 1940.

154. Samuels, L. T.; Ritz, N. D., and Poyet, E. B.: The Effect of Drugs on Vitamin C Excretion, J. Pharmacol. & Exper. Therap. **68**:465-475 (April) 1940.

to the animals' processes of detoxification. Ralli and Sherry [155] observed a decrease in both the concentration in plasma and the urinary excretion after injection of insulin into diabetic dogs. They were able to overcome this effect by the intravenous administration of dextrose.

Butler and Cushman [156] made an interesting and important contribution to the knowledge of the distribution of ascorbic acid in the blood. They described procedures for the determination of the ascorbic acid content of erythrocytes, leukocytes, platelets and whole blood. In the leukocytes they found an average value of 34 mg. of ascorbic acid equivalents per hundred grams of the white layer of centrifuged blood of normal persons and complete absence of the vitamin in the blood of persons with scurvy. These procedures may well prove to be a more accurate index of the vitamin C nutrition of the body than the determination of the vitamin C content of the plasma of fasting persons. Evidence was presented [157] which indicated that ascorbic acid passes from the plasma into the erythrocytes, its ratio of distribution varying with the state of ascorbic acid nutrition.

Methods Used in the Diagnosis of Deficiency.—In a previous review [158] it was noted that the simplest procedure for determining the state of ascorbic acid nutrition was estimation of ascorbic acid in the plasma after fasting. More recent evidence would indicate that this may not be an accurate index of the body's reserve of the vitamin. Several investigators [159] who used this method with healthy persons often found values within the "scorbutic range"; the plasma of many apparently healthy persons contained less than 0.1 mg. per hundred cubic centimeters. Snelling [159a] expressed the belief that there was "no

155. Ralli, E. P., and Sherry, S.: Effects of Insulin on Plasma Level and Excretion of Vitamin C, Proc. Soc. Exper. Biol. & Med. **43**:669-672 (April) 1940.

156. Butler, A. M., and Cushman, M.: Distribution of Ascorbic Acid in the Blood and Its Nutritional Significance, J. Clin. Investigation **19**:459-467 (May) 1940.

157. (*a*) Heinemann, M., and Hald, P. M.: Factors That Influence the Passage of Ascorbic Acid from Serum to Cells in Human Blood, J. Clin. Investigation **19**: 469-473 (May) 1940. (*b*) Butler and Cushman.[156]

158. Wilder, R. M.; Rutledge, D. I., and Wilbur, D. L.: Diseases of Metabolism and Nutrition: Review of Certain Recent Contributions, Arch. Int. Med. **63**:356-427 (Feb.) 1939.

159. (*a*) Snelling, C. E.: The Plasma Ascorbic Acid of Infants and Children, J. Pediat. **15**:824-830 (Dec.) 1939. (*b*) Yü, T. F.: Blood Vitamin C Content of Chinese in Peiping During the Winter of 1938 to 1939, Chinese M. J. **56**:334-344 (Oct.) 1939. (*c*) Mindlin, R. L.: Variations in the Concentration of Ascorbic Acid in the Plasma of the Newborn Infant, J. Pediat. **16**:275-284 (March) 1940. (*d*) Harris, L. J.: Assessment of the Level of Nutrition: Tests for Vitamin C on Groups of Poorly Fed and Well-Fed School-Children, Lancet **2**:259-263 (Aug. 31) 1940. (*e*) Minot, A. S.; Dodd, K.; Keller, M., and Frank, H.: A Survey of the State of Nutrition with Respect to Vitamin C in a Southern Pediatric Clinic, J. Pediat. **16**:717-728 (June) 1940.

justification in the use of this method for the diagnosis of scurvy." In large groups, there seemed to be a parallelism between the content of ascorbic acid in the plasma and the adequacy of the diet, but this was not necessarily true in individual cases.

Various methods which depend on the increased excretion or the elevated plasma levels following the administration of test doses of ascorbic acid have been described for the estimation of the state of ascorbic acid nutrition. Several authors [160] have concluded that these methods are superior to those which depend on fasting levels of plasma. The former may be criticized on the grounds that they reflect the body's saturation with ascorbic acid, which condition may not be entirely necessary to health. Ludden and Wright [161] devised a rather ingenious formula for a simplified method of determining the twenty-four hour excretion of ascorbic acid in the presence of renal insufficiency.

On finding low urinary excretion of ascorbic acid and normal levels in the plasma in cases of Addison's disease, Jenovese, Osterberg and Rynearson [162] concluded that determination of the ascorbic acid in the plasma alone is not an accurate index of deficiency of ascorbic acid in such cases.

The measurement of capillary fragility [163] and intradermal dye tests [164] have been rather conclusively demonstrated to be of little value.

New procedures continue to appear for the chemical determination of ascorbic acid in biologic materials and body fluids,[165] as well as methods which obviate sources of error in these determinations.[166]

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160. Kajdi, L.; Light, J., and Kajdi, C.: A Test for the Determination of the Vitamin C Storage: Vitamin C Index, J. Pediat. **15**:197-218 (Aug.) 1939. Richardson, J. E., and Mayfield, H. L.: The Amount of Ascorbic Acid Excreted at Each Urination During Twenty-Four-Hour Periods, Am. J. Physiol. **128**:583-587 (Feb.) 1940. Pemberton, J.: A Rapid Method of Differentiating Children with Large or Small Reserves of Vitamin C, Brit. M. J. **2**:217-219 (Aug. 17) 1940.

161. Ludden, J. B., and Wright, I.: Effect of Renal Retention of Vitamin C on Saturation Tests: A Formula for Compensation of This Factor of Error, Arch. Int. Med. **65**:151-162 (Jan.) 1940. Wright and Ludden.[150]

162. Jenovese, J. F.; Osterberg, A. E., and Rynearson, E. H.: Rôle of Vitamin C in Addison's Disease, Proc. Soc. Exper. Biol. & Med. **44**:335-339 (June) 1940.

163. Rapaport, H. G.; Miller, S. H., and Sicular, A.: Capillary Fragility and Ascorbic Acid Studies, J. Pediat. **16**:624-626 (May) 1940.

164. Rapaport, H. G., and Miller, S. H.: The Determination of Vitamin C in Children by Intradermal Injection, J. Pediat. **16**:503-507 (Oct.) 1939. Bakhsh, I.; Kochhar, B. D., and Malik, A. Q.: The Evaluation of the Intradermal Dye Test for Vitamin C in Health and Disease, Indian J. M. Research **27**:695-703 (Jan.) 1940.

165. Woessner, W. W.; Elvehjem, C. A., and Schuette, H. A.: The Determination of Ascorbic Acid in Commercial Milks, J. Nutrition **18**:619-626 (Dec.) 1939. Odin, M.: C-Vitaminstandard und C-Hypovitaminose: II. Bestimmung von Ascorbinsäure mittels der photoelektrischen Zelle, Acta pædiat. **26**:351-361, 1939.

(Footnotes continued on next page)

Data on Clinical Use.—The possibility that ascorbic acid may have a role in the healing of wounds after operations has attracted the attention of several investigators. Wolfer and Hoebel[167] presented an excellent historical summary of the healing of wounds in the presence of scurvy. The tendency to failure of healing under such a condition was observed by laymen two centuries ago. Among other things, Wolfer and Hoebel[167] reported that a postoperative decrease in the content of ascorbic acid in the blood occurred not infrequently. Various explanations were advanced, including (1) increased destruction of ascorbic acid, (2) increased utilization of ascorbic acid in healing, (3) increased storage of the vitamin and (4) abnormal function of the bowel. The long periods of intravenous therapy without intake of food by mouth which so commonly follow surgical procedures were mentioned by them as an important factor in this respect. They stated that many patients undergo operation who already have deficiency of ascorbic acid. They suggested that the following groups of patients should be investigated for the presence of such a condition: (1) those who are receiving a deficient diet, as ascertained from the history; (2) those who are receiving large doses of alkalis by mouth; (3) those who have obstructive lesions of the gastrointestinal tract; (4) those who have had long periods of vomiting (and they may well have added "or diarrhea"), and (5) those who have syphilis or are addicted to alcohol. They recommended that the patient who has deficiency of vitamin C be given 1 Gm. of ascorbic acid daily for ten days before operation is attempted and that this dose be continued after operation until healing is complete. Several cases were reported in which treatment with ascorbic acid seemed to be effective in bringing about proper healing of surgical wounds. Particularly striking was that of 1 patient (case 3 in Wolfer's[168] report) in whom secondary closure of an eviscerated wound was followed by prompt healing after administration of ascorbic acid.

Woessner, W. W.; Elvehjem, C. A., and Schuette, H.: The Determination of Ascorbic Acid in Evaporated Milk, Powdered Milk and Powdered Milk Products J. Nutrition **20**:327-338 (Oct. 10) 1940. Bryan, A. H.; Turner, D.; Lotwin, G., and Huenermann, R. L.: The Estimation of the Ascorbic Acid Content of the Diet, J. Am. Dietet. A. **16**:891-897 (Nov.) 1940. Heinemann and Hald.[157a]

166. Greenberg, L. D., and Rinehart, J. F.: The Stability of Ascorbic Acid in Blood, J. Lab. & Clin. Med. **25**:1288-1294 (Sept.) 1940. Sherry, S., and Friedman, G. J.: Recovery of Vitamin C from the Human Bladder, Proc. Soc. Exper. Biol. & Med. **42**:707-709 (Dec.) 1939. Kassan, R. J., and Roe, J. H.: The Preservation of Ascorbic Acid in Drawn Samples of Blood, J. Biol. Chem. **133**: 579-584 (April) 1940.

167. Wolfer, J. A., and Hoebel, F. C.: The Significance of Cevitamic Acid Deficiency in Surgical Patients, Surg., Gynec. & Obst. **69**:745-755 (Dec.) 1939.

168. Wolfer, J. A.: The Surgical Aspect of Vitamin-C Deficiency, S. Clin. North America **20**:225-240 (Feb.) 1940. Wolfer and Hoebel.[167]

Although it seems logical that dietary deficiency might be a factor in the failure of wounds to heal, Crandon and Lund [169] conducted an unusual experiment which tended to indicate that ascorbic acid avitaminosis may not be specific in this respect. By dietary measures they obtained total depletion of the ascorbic acid reserves in a healthy man as evidenced by complete absence of ascorbic acid in the blood plasma, leukocytes and platelets. Although they were unable to recover any ascorbic acid from a specimen of muscle removed for biopsy from the region of a wound produced for purposes of study, this wound healed as rapidly and firmly as that of a control subject; microscopic examination of the specimen failed to reveal any lack of intercellular substance. In a more recent report [170] of observations on this same person, clinical evidence of scurvy was noted after the diet had been completely lacking in ascorbic acid for one hundred and thirty-two days. When the blood levels of ascorbic acid had been zero for one hundred and forty-one days, another wound was made; definite failure of healing occurred, and the tissues revealed lack of intercellular substance and of formation of capillaries. The administration of ascorbic acid brought about good healing, and considerable intercellular substance appeared within ten days. From these observations it would appear that present methods of estimating the reserve of ascorbic acid are relatively crude.

In 1939, Holmes and associates [171] produced evidence that ascorbic acid was effective in the treatment and prevention of lead poisoning. Pillemer and his associates [172] were unable to substantiate this observation in experiments on guinea pigs and concluded that although treatment with this substance had some effect in the prevention of neuroplumbism, it was completely ineffective as compared with treatment by removal from exposure. Dannenberg and his co-workers [173] found ascorbic acid to be valueless as a therapeutic agent in a case of lead poisoning.

Farmer, Abt and Aron [174] demonstrated that the administration of arsenic and iron to human beings caused a marked decrease in the

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169. Crandon, J. H., and Lund, C. C.: Vitamin C Deficiency in an Otherwise Normal Adult, New England J. Med. **222**:748-752 (May 2) 1940.

170. Crandon, J. H.; Lund, C. C., and Dill, D. B.: Experimental Human Scurvy, New England J. Med. **223**:353 (Sept. 5) 1940. Experimental Human Scurvy, editorial, J. A. M. A. **115**:1637-1638 (Nov. 9) 1940.

171. Holmes, H. N.; Campbell, K., and Amberg, E. J.: The Effect of Vitamin C on Lead Poisoning, J. Lab. & Clin. Med. **24**:1119-1127 (Aug.) 1939.

172. Pillemer, L.; Seifter, J.; Kuehn, A. O., and Ecker, E. E.: Vitamin C in Chronic Lead Poisoning: An Experimental Study, Am. J. M. Sc. **200**:322-327 (Sept.) 1940.

173. Dannenberg, A. M.; Widerman, A. H., and Friedman, P. S.: Ascorbic Acid in the Treatment of Chronic Lead Poisoning: Report of a Case of Clinical Failure, J. A. M. A. **114**:1439-1440 (April 13) 1940.

174. Farmer, C. J.; Abt, A. F., and Aron, H. C. S.: Influence of Arsenicals, Bismuth and Iron on the Plasma Ascorbic Acid Level, Proc. Soc. Exper. Biol. & Med. **44**:495-499 (June) 1940.

amount of ascorbic acid in the plasma, whereas bismuth did not exert this effect. Of importance may be their observation that the administration of suitable doses of ascorbic acid to persons sensitive to arsenic permitted the resumption of treatment.

Further studies have continued to appear on the relation of deficiency of ascorbic acid to nutritional anemia, and the results have been conflicting. Aron [175] produced anemia in guinea pigs with diets deficient in ascorbic acid; this anemia was unaffected by administration of iron but responded to ascorbic acid. Lozner [176] demonstrated normal regeneration of hemoglobin in patients whose plasma did not contain any ascorbic acid.

Additional investigation of ascorbic acid nutrition in various infectious processes has added little to knowledge of this relation.[177] Among the conditions studied were pneumonia,[177c] rheumatoid arthritis [178] and pulmonary tuberculosis.[179] The general consensus was that the diminished quantity of ascorbic acid in the plasma, so frequently encountered in febrile and in chronic infectious conditions, is nonspecific and is dependent rather on an increased utilization or a general state of malnutrition.

Merritt and Foster [180] were unable to find any deviations from normal values in the content of ascorbic acid in the blood of patients who had epilepsy, nor did they observe any changes among those patients who were treated with dilantin sodium and exhibited hypertrophic gingivitis. They concluded that this condition is not due to a deficiency of ascorbic acid, as suggested by Kimball.[181]

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175. Aron, H. C. S.: The Relation of Vitamin C Deficiency to Nutritional Anemia, J. Nutrition **18**:375-383 (Oct.) 1939.

176. Lozner, E. L.: Studies on Hemoglobin Regeneration in Patients with Vitamin C Deficiency, J. Clin. Investigation **19**:787 (Sept.) 1940.

177. (*a*) Flexner, J.; Chassin, M., and Wright, I. S.: Studies on Herpes Simplex Encephalitis in Rabbits: I. The Therapeutic Effect of Vitamin C, Sulphanilamide and Pitressin, J. Infect. Dis. **66**:30-32 (Jan.-Feb.) 1940. (*b*) Abbasy, M. A.: On the Relation Between Vitamin C and Infection, J. Egyptian M. A. **23**:87-105 (Feb.) 1940. (*c*) Görtz, G.: Investigation of the Ascorbic Acid Excretion in the Urine of Healthy and Febrile Children, Acta pædiat. **27**: 429-436, 1940.

178. Jacques, R. H.: Relation Between Reduced Ascorbic-Acid Levels of the Blood Plasma and Rheumatoid Arthritis, J. Bone & Joint Surg. **22**:324-326 (April) 1940. Hall, M. G.; Darling, R. C., and Taylor, F. H. L.: The Vitamin C Requirement in Rheumatoid Arthritis, Ann. Int. Med. **13**:415-423 (Sept.) 1939.

179. Erwin, G. S.; Wright, R., and Doherty, C. J.: Hypovitaminosis-C and Pulmonary Tuberculosis, Brit. M. J. **1**:688-689 (April 27) 1940. Osborn, T. W. B., and Gear, J. H. S.: Possible Relation Between Ability to Synthesize Vitamin C and Reaction to Tubercle Bacillus, Nature, London **145**:974 (June 22) 1940.

180. Merritt, H. H., and Foster, A.: Vitamin C in Epilepsy: Dilantin Sodium Not a Cause of Vitamin C Deficiency, Am. J. M. Sc. **200**:541-544 (Oct.) 1940.

181. Kimball, O. P.: The Treatment of Epilepsy with Sodium Diphenyl Hydantoinate, J. A. M. A. **112**:1244-1245 (April 1) 1939.

Human Requirements.—The exact requirement of ascorbic acid in the diet of healthy persons is still unsettled. Most investigations indicated that the maximal intake necessary to produce saturation of the tissues is approximately 100 mg. each day, or 1.5 to 1.7 mg. per kilogram of body weight.[182] From the point of view of simple maintenance of good health, Fox and his associates [183] conducted an interesting experiment among mine workers. After supplementing the routine diet of these workers (which contained 12 to 25 mg. of ascorbic acid daily) with 40 mg. of ascorbic acid daily, they were unable to note any effect on changes in weight, physical efficiency or resistance to infectious or dental diseases.

Some speculation has arisen concerning the relative merits of the synthetic and the natural sources of ascorbic acid. Shepherd and her associates [143] found better retention of calcium, phosphorus and nitrogen during the use of ascorbic acid from natural sources, and Todhunter and associates [184] observed that ascorbic acid from natural sources was better than the synthetic product in prevention of hemorrhages in scorbutic guinea pigs. Variations [185] in blood plasma levels during the use of ascorbic acid from these different sources were not noted. It has been suggested that the juice of oranges and of lemons may contain an added antiscorbutic agent. Detrick and associates [186] did not find any evidence of this factor in citrin. In any event, these experiments seem to bear out the thesis that ascorbic acid from natural sources should be used whenever possible.

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182. Chen, K. C.; Yü, T. F.; Liu, S. H., and Chu, H. I.: Studies on the Vitamin C Requirement of Chinese Patients with Scurvy, Chinese J. Physiol. **15**: 119-142 (April 30) 1940. Todhunter, E. N., and Robbins, R. C.: Observations on the Amount of Ascorbic Acid Required to Maintain Tissue Saturation in Normal Adults, J. Nutrition **19**:263-270 (March) 1940. Ralli, E. P.; Friedman, G. J., and Sherry, S.: The Vitamin C Requirement of Man Estimated After Prolonged Studies of the Plasma Concentration and Daily Excretion of Vitamin C in Three Adults on Controlled Diets, J. Clin. Investigation **18**:705-714 (Nov.) 1939.

183. Fox, F. W.; Dangerfield, L. F.; Gottlich, S. F., and Jokl, E.: Vitamin C Requirements of Native Mine Labourers: An Experimental Study, Brit. M. J. **2**:143-147 (Aug. 3) 1940.

184. Todhunter, E. N.; Robbins, R. C.; Ivey, G., and Brewer, W.: A Comparison of the Utilization by Guinea Pigs of Equivalent Amounts of Ascorbic Acid (Vitamin C) in Lemon Juice and in the Crystalline Form, J. Nutrition **19**:113-120 (Feb.) 1940.

185. Todhunter, E. N., and Fatzer, A. S.: A Comparison of the Utilization by College Women of Equivalent Amounts of Ascorbic Acid (Vitamin C) in Red Raspberries and in Crystalline Form, J. Nutrition **19**:121-130 (Feb.) 1940. Todhunter, Robbins, Ivey and Brewer.[184]

186. Detrick, L. E.; Dunn, M. S.; McNamara, W. L., and Hubbard, M. E.: Vitamin C Studies: I. The Effect of Vitamin P (Citrin) on Vitamin C Deficient Guinea Pigs, J. Lab. & Clin. Med. **25**:684-687 (April) 1940.

VITAMIN D

Chemical and Physiologic Properties.—Liu and his associates [187] reported some interesting observations on the function of vitamin D in relation to the body's metabolism of calcium and phosphorus. They demonstrated that in cases of osteomalacia the absence of vitamin D interfered with the intestinal absorption of calcium and led to loss of this mineral. In carefully conducted studies, they found that the first sign of deficiency of vitamin D was the disappearance of calcium from the urine, which they interpreted as an attempt by the body to conserve this mineral. An increase of calcium in the stool followed; the quantity increased progressively with the deficiency until a negative calcium balance existed. The changes in metabolism of phosphorus differed only in that the urinary excretion of phosphorus increased. By adding as little vitamin D as that contained in one to two eggs to the diet each day, they were able to reverse these changes, and they observed that the effect of even these small doses was prolonged over many days. The metabolism of calcium and of phosphorus of nursing infants closely paralleled the aforementioned changes and was dependent on the content of vitamin D in the diet of the mother. Smith and Spector [188] presented additional evidence of the function of vitamin D in the absorption of calcium, since they were able to show that the addition of liquid petrolatum to the diet of rats interfered with the healing of rickets by ultraviolet irradiation. Cohn and Greenberg,[189] in studying the metabolism of radioactive phosphorus in rachitic rats, found the intestinal absorption of this element little increased by vitamin D, but did observe that the vitamin increased the absorption of inorganic phosphorus by bone from 25 to 50 per cent. Reasoning indirectly, they suggested that vitamin D may exert its action through the conversion of organic to inorganic phosphorus in the bone.

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187. Liu, S. H.: The Role of Vitamin D in the Calcium Metabolism in Osteomalacia, Chinese M. J. **57**:101-118 (Feb.) 1940. Liu, S. H.; Chu, H. I.; Su, C. C.; Yu, T. F., and Cheng, T. Y.: Calcium Phosphorus Metabolism in Osteomalacia: IX. Metabolic Behavior in Infants Fed on Breast Milk from Mothers Showing Various States of Vitamin D Nutrition, J. Clin. Investigation **19**:327-347 (March) 1940. Chu, H. I.; Liu, S. H.; Yu, T. F.; Hsu, H. C.; Cheng, T. Y., and Chao, H. C.: Calcium and Phosphorus Metabolism in Osteomalacia: X. Further Studies on Vitamin D Action; Early Signs of Depletion and Effect of Minimal Doses, ibid. **19**:349-363 (March) 1940.

188. Smith, M. C., and Spector, H.: Further Evidence of the Mode of Action of Vitamin D, J. Nutrition **20**:197-202 (Sept. 10) 1940.

189. Cohn, W. E., and Greenberg, D. M.: Studies in Mineral Metabolism with the Aid of Artificial Radioactive Isotopes: III. The Influence of Vitamin D on the Phosphorus Metabolism of Rachitic Rats, J. Biol. Chem. **130**:625-634 (Oct.) 1939.

Sobel and his associates [190] made the interesting observation that in rats lead seemed to be deposited in the bones in a manner similar to that of calcium, and that lead, phosphorus and vitamin D formed a system for deposition of lead which appeared to be analogous to that for calcification. They demonstrated also that administration of vitamin D had the same effect on the content of lead in the blood as did the administration of calcium.

Lichtenstein [191] demonstrated definite bactericidal power in the cod liver oil used for dressings and proved that this was closely related to its content of peroxides.

Successful synthesis of compounds related to the antirachitic vitamins was reported,[192] and it is hoped that a suitable product will soon be available. New methods for the determination of vitamin D activity of foods and biologic materials appeared.[193] Warkany and Mabon,[193d] who used a method of biologic assay employing rachitic rats, found the serum of normal persons to contain between 66 and 165 U. S. P. units of vitamin D per hundred cubic centimeters of blood.

Treatment of Rickets.—Park [194] presented a complete review of the treatment of rickets and infantile tetany with vitamin D. For preventive measures he emphasized the importance of commencing early and reaching the full dose "certainly by the end of the second month" of life. He warned that in the active treatment of rickets milk containing vitamin D

190. Sobel, A. E.; Yuska, H.; Peters, D. D., and Kramer, B.: The Biochemical Behavior of Lead: I. Influence of Calcium, Phosphorus and Vitamin D on Lead in Blood and Bone, J. Biol. Chem. **132**:239-265 (Jan.) 1940.

191. Lichtenstein, M.: Cod-Liver-Oil Dressings: Their Mode of Action, Lancet **2**:1023-1026 (Nov. 11) 1939.

192. Milas, N. A., and Alderson, W. L., Jr.: Studies in the Synthesis of the Anti-Rachitic Vitamins: I. The Synthesis of 3-[2′-Methylenecyclohexylidene-1′]-propene-1, J. Am. Chem. Soc. **61**:2534-2537 (Sept.) 1939. Aldersley, J. B.; Burkhardt, G. N.; Gillam, A. E., and Hindley, N. C.: The Synthesis of Compounds Related to the Antirachitic Vitamins: Part II, J. Chem. Soc. London, January 1940, pp. 10-16.

193. (*a*) Westerlung, A.: A Biological Estimation of Vitamin D in Oils, Upsala läkaref. förh. **45**:33-45, 1939. (*b*) Coward, K. H., and Kassner, E. W.: The Determination of Vitamin D in Food Substances Containing Phosphorus, Biochem. J. **34**:538-541 (April) 1940. (*c*) Russell, W. C.: Report on Vitamin D: Feeding of Non-Vitamin D Skim or Whole Milk with the Reference to Cod Liver Oil, J. A. Off. Agric. Chem. **23**:341-345 (May) 1940. (*d*) Warkany, J., and Mabon, H. E.: Estimation of Vitamin D in Blood Serum: II. Level of Vitamin D in Human Blood Serum, Am. J. Dis. Child. **60**:606-614 (Sept.) 1940. (*e*) McChesney, E. W., and Homburger, E.: A Modification of the Line Test Applicable to Chicken Vitamin D Assay, J. Nutrition **20**:339-349 (Oct. 10) 1940. (*f*) Nield, C. H.; Russell, W. C., and Zimmerli, A.: The Spectrophotometric Determination of Vitamins D_2 and D_3, J. Biol. Chem. **136**:73-79 (Oct.) 1940.

194. Park, E. A.: The Therapy of Rickets, J. A. M. A. **115**:370-379 (Aug. 3) 1940.

did not exhibit sufficient activity to end the disease abruptly; he recommended the use of the various fish oil preparations in doses sufficient to furnish 1,000 U. S. P. units of vitamin D daily, or even 10,000 to 20,000 units to premature infants. Attention was called to the essential role of calcium in the treatment of infantile tetany. Park [194] reasoned properly that the use of vitamin D alone in this condition was irrational. He included a comprehensive discussion of the use of sunlight and ultraviolet irradiation, the treatment of refractory rickets and the signs of toxicity from vitamin D therapy; a summary of the various sources of vitamin D and the relative costs of these different preparations completed an excellent presentation of this subject, which is highly recommended as a "must read" article to any physician who treats children in his practice.

Since the elaboration of *Vitaminstoss* (crystalline vitamin D_2) therapy by Braulke [195] in 1937, the great bulk of antirachitic investigation has been in this direction. Parenteral [196] administration of 500,000 to 1,000,000 U. S. P. units of vitamin D to children who had rickets (including premature infants) was followed by uniformly rapid healing and complete absence of clinical evidence of toxicity. This method seems to have been given a clinical trial sufficiently thorough to establish it as a standard procedure. Vollmer [196c] demonstrated more rapid healing in rachitic rabbits by administering crystalline vitamin D dissolved in ether and oil (0.4 cc. of ether and 0.6 cc. of peanut oil) than by employing only oil as the solvent. Zelson [196d] proposed a plan (suggested by Vollmer) for the prevention of rickets by parenteral vitamin D "shock prophylaxis," which appears to possess many advantages over the present methods.

The relative antirachitic properties of vitamins D_2 and D_3 has received some attention, to the ultimate conclusion that no significant clinically effective difference exists between the two preparations.[197]

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195. Braulke, H.: Die Indikationen der Rachitisbehandlung mit einmaliger Dosis von Vitamin D_2 (Vitaminstoss), Ztschr. f. Kinderh. **59**:18-31, 1937; abstracted, Am. J. Dis. Child. **56**:643-644 (Sept.) 1938.

196. (*a*) Gunnarson, S.: Treatment of Rickets with a Single Massive Dose of Vitamin D_2, Acta pædiat. **25**:69-81, 1939. (*b*) Ström, J.: The Treatment of Spasmophilia with a Single Massive Dose of Vitamin D_2, ibid. **25**:251-265, 1939. (*c*) Vollmer, H.: Treatment of Rickets and Tetany by Parenteral Administration of One Massive Dose of Vitamin D, J. Pediat. **16**:419-432 (April) 1940. (*d*) Zelson, C.: Prevention of Rickets in Premature Infants with Parenteral Administration of Single Massive Doses of Vitamin D, ibid. **17**:73-78 (July) 1940.

197. Morris, N., and Stevenson, M. M.: Vitamins D_2 and D_3 in Infantile Rickets: A Comparison of Their Therapeutic Efficiency, Lancet **2**:876-879 (Oct. 21) 1939. Himsworth, H. P., and Maizels, M.: Vitamins D_2 and D_3 and A. T. 10 in Congenital Thyroid and Parathyroid Deficiency, ibid. **1**:959-960 (May 25) 1940. Wilson, D. C.: Potency of Vitamins D_2 and D_3 in Osteomalacia and Late Rickets, ibid. **1**:961-962 (May 25) 1940. Park.[194]

As Park [194] pointed out, "One need not fear that the dosage of vitamin D will be toxic unless renal insufficiency exists or unless the dosage is extremely large." This statement seems to be well corroborated by observations on clinical "shock therapy," [196] and animal intoxication has, with 1 exception,[198] been produced only by doses many times larger than those used clinically.[199] Vollmer [196c] demonstrated the presence of toxic by-products in irradiated ergosterol and suggested that the crystalline vitamins may be more safely employed if large doses are given. It was his opinion that an infant who has rickets possesses a definitely greater tolerance for vitamin D than does a nonrachitic adult.

Treatment of Other Conditions.—Knapp [200] offered experimental evidence to indicate that vitamin D is a possible factor in progressive myopia. He was able to reduce myopia or check its progression in two thirds of his patients. Reports of the use of vitamin D in various diseases of the skin [201] are fragmentary and offer little of value.

Poer [202] treated 11 patients who had postoperative hypoparathyroid tetany and expressed the opinion that over a prolonged period vitamin D_2 and dihydrotachysterol are more effective than parathyroid extract.

VITAMIN E

Chemical and Physiologic Properties.—Recent advances in the knowledge of vitamin E have been confined in large measure to the field of chemistry. Details of this advance may be found in journals or books devoted to this subject.[203] Evans and his associates [204] expressed the

198. Chown, B.; Lee, M.; Teal, J., and Currie, R.: On the Experimental Production of Nephritis in Rats by Means of Parathyroid Hormone and of Vitamin D, J. Path. & Bact. **49**:273-290 (Sept.) 1939.

199. Harris, R. S.; Ross, B. D., and Bunker, J. W. M.: Histological Study of Hypervitaminosis D: The Relative Toxicity of Vitamin D of Irradiated Ergosterol and Tuna Liver Oil, Am. J. Digest. Dis. **6**:81-83 (April) 1939.

200. Knapp, A. A.: Vitamin-D Complex in Progressive Myopia: Etiology, Pathology, and Treatment; Preliminary Study, A. Research Ophth., Proc. **10**:80-88, 1939; Vitamin-D Complex in Progressive Myopia: Etiology, Pathology and Treatment; Preliminary Study, Am. J. Ophth. **22**:1329-1337 (Dec.) 1939.

201. Simpson, C. A.; Ellis, F. A., and Kirby-Smith, H.: Vitamin D in the Treatment of Acne, Arch. Dermat. & Syph. **41**:835-837 (May) 1940. Maynard, M. T. R.: Vitamin Therapy in Dermatology with Particular Reference to Vitamin D in the Treatment of Acne and of Diseases Due to Altered Usage of Calcium, ibid. **41**:842-857 (May) 1940. Thacker, E. A.: The Treatment of Psoriasis with Various Vitamin D Preparations, Illinois M. J. **78**:352-360 (Oct.) 1940.

202. Poer, D. H.: Dihydrotachysterol, Parathormone and Vitamin D_2: Comparison of Their Values in the Treatment of Post-Thyroidectomy Hypocalcemic Tetany, South. M. J. **33**:1174-1180 (Nov.) 1940.

203. Smith, L. I.: The Chemistry of Vitamin E, Chem. Rev. **27**:287-329 (Oct.) 1940; Vitamin E, New York, Chemical Publishing Co., Inc., 1940.

(Footnotes continued on next page)

belief that the following structural properties are necessary for the activity of vitamin E: (1) a certain structural skeleton which will permit ready conversion of the substance to an oxidation-reduction system, and (2) accessory groups of such a nature as to provide solubility for absorption and transportation. Furthermore, the organism must have the ability to effect the necessary chemical transformation. Karrer [205] demonstrated the importance in biologic activity of the long aliphatic side chain and methyl substitution in the aromatic nucleus. The tocopherol esters were shown to possess vitamin E activity equal to that of the parent tocopherol, with the added advantage that they are more stable to oxidation.[206]

Numerous procedures have been devised for the chemical estimation of the tocopherols, but most of these methods have the same fundamental shortcoming in that they do not distinguish the various tocopherols that differ in biologic activity.

In contrast to the remarkable strides in the chemical field, little has been added to the knowledge of the physiologic role of vitamin E in living organisms. Progress has been hampered by many experimental difficulties, and results and conclusions are not in agreement. As has been found to be true in regard to other vitamins, the possibility seems to exist that the tocopherols and their homologues may possess some function in the general cellular metabolism of the animal body, but this has not been proved. Pappenheimer [207] listed the nutritional disorders of various laboratory animals deficient in vitamin E as (1) nutritional encephalomalacia of chicks, (2) nutritional myopathy of ducklings, (3) nutritional myopathy of the gizzard of turkeys, (4) nutritional muscular dystrophy of guinea pigs and rabbits and (5) muscular dystrophy of young rats. He expressed the belief that whereas no forecast can be made regarding the relation of these conditions to human disorders, there is enough evidence "to justify a cautious empiricism." He suggested as one of the significant lessons from his work the fact that a partial deficiency of vitamin E in the mother may manifest itself only in the offspring. He suggested further that this fact may help to explain

204. Evans, H. M.; Emerson, O. H.; Emerson, G. A.; Smith, L. I.; Ungnade, H. E.; Prichard, W. W.; Austin, F. L.; Hoehn, H. H.; Opie, J. W., and Wawzonek, S.: The Chemistry of Vitamin E: XIII. Specificity and Relationship Between Chemical Structure and Vitamin E Activity, J. Organ. Chem. **4**:376-388 (Sept.) 1939.

205. Karrer, P.: Vitamin E und verwandte Verbindungen, Helvet. chim. acta **22**:334-360, 1939.

206. Demole, V.; Isler, O.; Ringier, B. H.; Salomon, H., and Karrer, P.: Ueber Ester des α-Tocopherols, Helvet. chim. acta **22**:65-68, 1939.

207. Pappenheimer, A. M.: Certain Nutritional Disorders of Laboratory Animals Due to Vitamin E Deficiency, J. Mt. Sinai Hosp. **7**:65-76 (July-Aug.) 1940.

the observed familial and hereditary characteristics of certain nervous and muscular disorders of human beings.

Results of animal experiments during the past year have added little to Pappenheimer's [207] list, and a good share of the work has been related to the vitamin E activity of various tocopherols and their derivatives. Pappenheimer, Goettsch and Karsubova [208] were able to prevent muscular dystrophy in young rats by section of the peripheral nerves; subsequent experiments by the same investigators revealed that section of the achilles tendon was equally effective in preventing dystrophy of the gastrocnemius muscle and that the loss of muscle tonus rather than the loss of the nerve supply itself appeared to afford protection. Elvehjem and his associates [209] produced muscular dystrophy among dogs by a diet deficient in vitamin E, but only when the animals were under the added physical strain of gestation and lactation.

The relation of vitamin E to the reproductive organs of laboratory animals has been recognized for several years; no recent contributions on the relation of the various conditions produced by lack of vitamin E to human sterility have appeared. Results of experimental work attempting to relate deficiency of vitamin E to dysfunction of the pituitary body are at variance and permit no conclusions.[210]

Evans [204] raised the following questions, which must be answered before the physiologic role of vitamin E can be understood:

1. What is the "specificity" of chemical structure in vitamin E responses, and are different portions of the tocopherol molecule necessary for neuromuscular and reproductive normality? 2. What is the actual mode of action of the vitamin in the physiology of embryos, seminiferous epithelium and neuromuscular apparatus? 3. What is the cause of death in E-free sucklings and how does spontaneous recovery ensue? 4. What analogous human clinical conditions exist either of myogenic or neurogenic origin?

Clinical Use.—During the past year, clinicians have concerned themselves with the possible benefit of vitamin E to various neuromuscular disorders. Bicknell [211] reported satisfactory improvement in cases of

208. Pappenheimer, A. M.; Goettsch, M., and Karsubova, C.: Effect of Nerve Section upon Development of Nutritional Muscular Dystrophy in Young Rats, Proc. Soc. Exper. Biol. & Med. **43**:313-316 (Feb.) 1940.

209. Anderson, H. D.; Elvehjem, C. A., and Gonce, J. E., Jr.: Vitamin E Deficiency in Dogs, Proc. Soc. Exper. Biol. & Med. **42**:750-755 (Dec.) 1939.

210. Koneff, A. A.: Pituitary Changes in Male Rats Reared and Maintained on "Pure" Dietaries With and Without Vitamin E, Anat. Rec. **74**:383-399 (Aug. 25) 1939. Drummond, J. C.; Noble, R. L., and Wright, M. D.: Studies on the Relationship of Vitamin E (Tocopherols) to the Endocrine System, J. Endocrinol. **1**:275-286 (Nov.) 1939.

211. Bicknell, F.: Vitamin E in the Treatment of Muscular Dystrophies and Nervous Diseases, Lancet **1**:10-13 (Jan. 6) 1940.

amyotrophic lateral sclerosis and progressive muscular dystrophy. Wechsler [212] treated 2 patients who had early amyotrophic lateral sclerosis and observed excellent results. Spies and Vilter [213] reported improvement in occasional cases of amyotrophic lateral sclerosis in which synthetic alpha tocopherol was used. Stone [214] noted definite improvement in 5 cases of muscular dystrophy and in several cases of muscular atrophy after wheat germ oil was added to the diet; he stated that addition of the vitamin B complex enhanced the therapeutic value of vitamin E. Vilter and his associates [122] reported more spectacular remissions in a case of acute arsenical peripheral neuritis after treatment with both pyridoxine (vitamin B_6) and alpha tocopherol than when the former was used alone.

In contradiction to these observations, Shelden, Woltman and one of us (H. R. B.) [215] were unable to demonstrate any improvement in 8 patients with progressive muscular dystrophy, 6 with amyotrophic lateral sclerosis and 4 with progressive muscular atrophy. These patients were treated for three to six months with combined wheat germ oil and synthetic alpha tocopherol. It is felt that further clinical evidence is needed to support the contention that lack of vitamin E is a primary factor in the causation of these various disorders.

The use of vitamin E in the treatment of various disorders of the human reproductive system was thoroughly discussed in a report of the Council on Pharmacy and Chemistry of the American Medical Association,[216] and the reader is referred to this publication for details of this subject. Some of the conclusions follow:

> Claims that vitamin E (wheat germ oil) is of value in the treatment of menstrual disorders, failure of lactation and the vaginal pruritus after the menopause . . . [and] in the prevention of habitual abortion cannot be accepted because of lack of convincing clinical evidence. . . . The published results of the treatment of habitual abortion with vitamin E are sufficiently encouraging to justify further clinical experiment.

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212. Wechsler, I. S.: Recovery in Amyotrophic Lateral Sclerosis Treated with Tocopherols (Vitamin E): Preliminary Report, J. A. M. A. **114**:948-950 (March 16) 1940.

213. Spies, T. D., and Vilter, R. W.: A Note on the Effect of Alpha-Tocopherol (Vitamin E) in Human Nutrition, South. M. J. **33**:663-664 (June) 1940.

214. Stone, S.: Treatment of Muscular Dystrophies and Allied Conditions: Preliminary Report on Use of Vitamin E (Wheat Germ Oil), J. A. M. A. **114**: 2187-2191 (June 1) 1940.

215. Shelden, C. H.; Butt, H. R., and Woltman, H. W.: Vitamin E (Synthetic Alpha-Tocopherol) Therapy in Certain Neurologic Disorders, Proc. Staff Meet., Mayo Clin. **15**:577-580 (Sept. 11) 1940.

216. The Treatment of Habitual Abortion with Vitamin E, report of the Council on Pharmacy and Chemistry, J. A. M. A. **114**:2214-2218 (June 1) 1940.

VITAMIN K

Chemical and Physiologic Properties.—Vitamin K_1 is a synthetic compound identical with 2-methyl-1, 4-naphthoquinone except that the vitamin bears a phytyl side chain in the 3 position (2-methyl-3-phytyl-1,4-naphthoquinone). The synthetic product is also identical with natural vitamin K_1, which is obtained from alfalfa,[217] although it is not utilized so rapidly by the body as 2-methyl-1, 4-naphthoquinone.[218] The correct empiric formula now proposed for vitamin K_2 is $C_{41}H_{60}O_2$.[219]

Most of the advances in the chemistry of vitamin K have been made in the study of naphthoquinone derivatives. Of those investigated, 2-methyl-1,4-naphthoquinone has proved to possess the most marked antihemorrhagic activity.[220] Ansbacher and Fernholz [221] were the first to show that this compound is as active as vitamin K, and this work now has been amply confirmed. In an extensive study of substituted alpha naphthoquinones, Binkley and his co-workers [222] found that the degree of activity of these substances varied widely, the highest activity being about a fifth that of pure vitamin K_1. Doisy and associates [223] reported that only 1,4-naphthoquinone and compounds which on oxidation in the organism might yield 1,4-naphthoquinone have vitamin K activity. Besides these authors, others [224] have isolated several water-soluble compounds which have a high antihemorrhagic activity.

Little is known about the physiologic effect of vitamin K. It is well established that it plays some vital role in the metabolism of prothrombin, but its exact mode of action is unknown.

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217. Fieser, L. F.: Synthesis of Vitamin K_1, J. Am. Chem. Soc. **61**:3467-3475 (Dec.) 1939. Emmett, A. D.; Brown, R. A., and Kamm, O.: Comparison of the Antihemorrhagic Activity of Natural and Synthetic Vitamin K_1 with the Proposed Standard 2-Methyl-1,4-Naphthoquinone, J. Biol. Chem. **132**:467-468 (Jan.) 1940.

218. Ansbacher, S.: Vitamin K, J. Biol. Chem. **133**:iii-iv (May) 1940.

219. Fieser, L. F.: Synthesis of 2-Methyl-3-Phytyl-1,4-Naphthoquinone, J. Am. Chem. Soc. **61**:2559-2561 (Sept.) 1939. Binkley, S. B.; McKee, R. W.; Thayer, S. A., and Doisy, E. A.: Structure of Vitamin K_2, J. Biol. Chem. **133**:xii-xiii (May) 1940.

220. Almquist, H. J., and Klose, A. A.: Antihemorrhagic Activity of 2-Methyl-1,4-Naphthoquinone, J. Biol. Chem. **130**:787-789 (Oct.) 1939.

221. Ansbacher, S., and Fernholz, E.: Simple Compounds with Vitamin K Activity, J. Am. Chem. Soc. **61**:1924-1925 (July) 1939.

222. Binkley, S. B.; Cheney, L. C.; Holcomb, W. F.; MacCorquodale, D. W.; Thayer, S. A., and Doisy, E. A.: The Vitamin K Activity of Some Substituted α-Naphthoquinone and of Some Related Compounds, abstracts read before the Division of the Biological Chemistry of the American Chemical Society, Boston, Sept. 11-15, 1939.

223. Doisy, E. A; MacCorquodale, D. W.; Thayer, S. A.; Binkley, S. B., and McKee, R. W.: The Isolation, Constitution and Synthesis of Vitamin K_1, Science **90**:407 (Nov. 3) 1939.

224. Moore, M. B., and Kirchmeyer, F. J.: Personal communication to the authors.

Among human beings serious untoward reactions have not been observed after reasonable therapeutic doses of either natural or synthetic compounds of vitamin K. As much as 5 mg. of 4-amino-2-methyl-1-naphthol hydrochloride has been administered intravenously in a single dose, and 40 mg. has been administered intravenously to human beings within a period of nineteen days without the occurrence of any untoward reaction. In a period of thirty-one days, 40 mg. of 2-methyl-1,4-naphthohydroquinone-3-sodium sulfonate was administered intravenously and 28 mg. administered orally to 1 person without causing any untoward reaction, either during or after its administration.[225] On the other hand, Koller [226] observed that large doses (180 mg.) of 2-methyl-1,4-naphthoquinone administered orally to human beings resulted in vomiting and porphyrinuria. When 30 mg. of this substance per kilogram of body weight was injected intramuscularly into a dog, vomiting, porphyrinuria and albuminuria resulted. Molitor and Robinson [227] also studied the acute and chronic toxicity of orally and parenterally administered vitamin K_1, phthiocol and 2-methyl-1,4-naphthoquinone. The oral lethal dose of phthiocol for mice was approximately 0.2 Gm. per kilogram of body weight, and that of 2-methyl-1,4-naphthoquinone, 0.5 Gm. per kilogram of body weight. In experiments conducted to determine chronic toxicity, it was noted that daily administration for thirty consecutive days of 0.35 Gm. of phthiocol and of 0.5 Gm. of 2-methyl-1,4 naphthoquinone per kilogram of body weight produced toxic reactions and that doses of 0.1 Gm. of phthiocol and 0.35 Gm. of 2-methyl-1,4-naphthoquinone per kilogram of body weight produced a marked decrease in the number of erythrocytes and the concentration of hemoglobin. Such effects were not observed after the administration of vitamin K_1.

It is now well established that a proper response to vitamin K depends on the integrity of the hepatic parenchyma. This was early established by Warner [228] and by one of us (H. R. B.) and associates.[229] Rhoads [230] found that 15 per cent of a group of patients who had hepatic damage did not respond to the administration of synthetic substances which had vitamin K activity. At necropsy severe hepatic damage was

225. Butt, H. R.; Snell, A. M., and Osterberg, A. E.: Unpublished data.

226. Koller, F.: Ueber die klinische Wirksamkeit von Naphtochinonderivaten (Vitamin K-Wirkung), Schweiz. med. Wchnschr. **69**:1159-1161 (Nov. 11) 1939.

227. Molitor, H., and Robinson, H. J.: Oral and Parenteral Toxicity of Vitamin K_1, Phthiocol and 2 Methyl 1,4 Naphthoquinone, Proc. Soc. Exper. Biol. & Med. **43**:125-128 (Jan.) 1940.

228. Warner, E. D., in discussion on Butt, Snell and Osterberg.[229]

229. Butt, H. R.; Snell, A. M., and Osterberg, A. E.: Oral and Intramuscular Administration of Vitamin K in Hemorrhagic Diathesis of Obstructive Jaundice, J. A. M. A. **112**:879-880 (March 4) 1939.

230. Rhoads, J. E.: Physiological Factors Regulating the Level of the Plasma Prothrombin, Ann. Surg. **111**:916 (May) 1940.

found. Bollman and his associates [231] noted that rats whose livers were severely injured by chloroform intoxication did not respond in the usual manner to treatment with vitamin K. Similar observations were reported by others.[232]

Methods of Measuring Prothrombin.—The measurement of prothrombin in the circulating blood is still a prerequisite to the intelligent use of vitamin K. In an excellent review of the literature on prothrombin in the plasma and vitamin K, Brinkhous [233] discussed in detail the various methods for measuring prothrombin. During the past year, the necessity of measuring the prothrombin in small quantities of blood obtained from newborn or premature infants has led to the development of several micromethods.[234]

Prothrombin in Stored Blood.—The level of prothrombin in stored blood has received considerable attention. It has been observed that citrated blood which is allowed to stand for any length of time ("bank blood") does not contain much prothrombin and is therefore of little value in the control of hemorrhage resulting from a deficiency of prothrombin.[235] In contrast to this observation, Belk and his associates [236] found that Quick's prothrombin time of stored blood was moderately prolonged after ten days, but not after five days. In contrast, Lord and Pastore [237] and Warner and his associates,[238] using the quantitative

231. Bollman, J. L.; Butt, H. R., and Snell, A. M.: The Influence of the Liver on the Utilization of Vitamin K, J. A. M. A. **115**:1087-1091 (Sept. 28) 1940.

232. Brinkhous, K. M., and Warner, E. D.: Effect of Vitamin K on Hypoprothrombinemia of Experimental Liver Injury, Proc. Soc. Exper. Biol. & Med. **44**: 609-610 (June) 1940.

233. Brinkhous, K. M.: Plasma Prothrombin: Vitamin K, Medicine **19**:329-416 (Sept.) 1940.

234. (*a*) Kato, K.: Micro-Prothrombin Test with Capillary Whole Blood: A Modification of Quick's Quantitative Method, Am. J. Clin. Path. **10**:147-153 (Feb.) 1940. (*b*) Quick, A. J.: Determination of Prothrombin, Proc. Soc. Exper. Biol. & Med. **42**:788-789 (Dec.) 1939. (*c*) Bray, W. E., and Kelley, O. R.: Prothrombin Studies, Especially in the Newborn, Am. J. Clin. Path. **10**:154-167 (Feb.) 1940. (*d*) Kelley, O. R., and Bray, W. E.: Prothrombin Time Determinations, J. Lab. & Clin. Med. **25**:527-530 (Feb.) 1940. (*e*) Kato, K., and Poncher, H. G.: The Prothrombin in the Blood of Newborn Mature and Immature Infants as Determined by the Micro Prothrombin Test, J. A. M. A. **114**:749-753 (March 2) 1940.

235. Rhoads, J. E., and Panzer, L. M.: The Prothrombin Time of "Bank Blood," J. A. M. A. **112**:309-310 (Jan. 28) 1939.

236. Belk, W. P.; Henry, N. W., and Rosenstein, F.: Observations on Human Blood Stored at Four to Six Degrees Centigrade, Am. J. M. Sc. **198**:631-633 (Nov.) 1939.

237. Lord, J. W., Jr., and Pastore, J. B.: Plasma Prothrombin Content of Bank Blood, J. A. M. A. **113**:2231-2232 (Dec. 16) 1939.

238. Warner, E. D., and Flynn, J. E.: Absorption of Water-Soluble Vitamin K from Intestinal Tract, Proc. Soc. Exper. Biol. & Med. **44**:607-608 (June) 1940.

prothrombin method of Warner, Brinkhous and Smith, observed that in stored blood maintained aseptically at 5 C. disintegration of plasma prothrombin occurred gradually; a value of 50 per cent of normal was reached at the end of about three weeks. Similar results were also obtained by Ziegler and her associates.[239]

Clinical Use of Vitamin K.—Numerous reports have appeared during the past year which tend to establish the fact that vitamin K in the form of natural concentrates or pure synthetic products which exhibit antihemorrhagic activity is useful in the prevention and control of the hemorrhagic diathesis that frequently occurs among patients who exhibit hypoprothrombinemia.

The commercial availability of synthetic compounds which possess antihemorrhagic activity has led to the general use of these materials, in many instances as a substitute for the formerly employed concentrates of alfalfa and of fish meal. Several investigators [240] reported the successful use of 2-methyl-1,4-naphthoquinone administered orally, intramuscularly and intravenously. In most cases, 1 to 4 mg. of this compound, together with 5 to 10 grains (0.3 to 0.65 Gm.) of animal bile salts, constituted an adequate daily oral dose. By the intravenous route, 1 to 2 mg. was likewise an effective daily dose in most instances. The results as judged on the basis of all these reports are good except in those instances in which severe hepatic damage was present.

In the hands of most investigators,[241] a water-soluble compound, 4-amino-2-methyl-1-naphthol hydrochloride, has proved to be just as effective when given by the intravenous route as the derivative of

239. Ziegler, E. R.; Osterberg, A. E., and Hovig, M.: The Prothrombin Changes in Banked Blood, J. A. M. A. **114**:1341-1342 (April 6) 1940.

240. MacCorquodale, D. W.; Cheney, L. C.; Binkley, S. B.; Holcomb, W. F.; McKee, R. W.; Thayer, S. A., and Doisy, E. A.: The Constitution and Synthesis of Vitamin K_1, J. Biol. Chem. **131**:357-370 (Nov.) 1939. Macfie, J. M.; Bacharach, A. L., and Chance, M. R. A.: The Vitamin K Activity of 2-Methyl-1:4-Naphthoquinone and Its Clinical Use in Obstructive Jaundice, Brit. M. J. **2**:1220-1223 (Dec. 23) 1939. Butt, H. R.; Snell, A. M.; Osterberg, A. E., and Bollman, J. L.: Treatment of Hypoprothrombinemia: Use of Various Synthetic Compounds Exhibiting Antihemorrhagic Activity (Vitamin K_1 Activity), Proc. Staff Meet., Mayo Clin. **15**:69-73 (Jan. 31) 1940. Rhoads, J. E., and Fliegelman, M. T.: The Use of 2-Methyl-1,4-Naphthoquinone (a Synthetic Vitamin K Substitute) in the Treatment of Prothrombin Deficiency in Patients, J. A. M. A. **114**:400-401 (Feb. 3) 1940. Sharp, E. A.: Vitamin K Activity of 2-Methyl-1,4-Naphthoquinone, ibid. **114**:439-440 (Feb. 3) 1940.

241. (*a*) Wilkie, D. P. D.: Surgery of the Biliary Passages, Tr. Med.-Chir. Soc. Edinburgh, 1924-1925, pp. 50-64; in Edinburgh M. J., March 25, 1925. (*b*) Emmett, A. D.; Kamm, O., and Sharp, E. A.: The Vitamin K Activity of 4-Amino-2-Methyl-1-Naphthol and 4-Amino-3-Methyl-1-Naphthol, J. Biol. Chem. **133**:285-286 (March) 1940. (*c*) Aggeler, P. M.; Lucia, S. P., and Goldman, L.: Effect of Synthetic Vitamin K Compounds on Prothrombin Concentration in Man. Proc. Soc. Exper. Biol. & Med. **43**:689-694 (April) 1940.

2-methyl-1,4-naphthoquinone. In Butt and Snell's experience,[242] this compound given intravenously in doses of 1 to 2 mg. has proved to be effective in reducing an elevated prothrombin clotting time. Untoward reactions have not followed its administration. As this compound is water soluble, the belief has been expressed that it may be administered orally without bile salts in the presence of biliary obstruction. Reports indicate that this can be done successfully both experimentally [243] and clinically.[243]

It is now well established that in certain cases of severe hepatic damage response to vitamin K does not occur.[244] It was observed that [245] in cases of primary hepatic disease the prothrombin clotting time is not often more than forty-five to fifty seconds. To reduce this elevated prothrombin time to within normal limits often is a hopeless task, regardless of the amounts of vitamin K administered. It is well to remember that patients who have severe primary hepatic damage constitute a group who are perhaps not primarily deficient in vitamin K but whose livers have lost the ability to produce prothrombin even in the presence of adequate amounts of vitamin K. In our own experience, patients with an increased prothrombin time which does not respond to the administration of vitamin K must be observed carefully after any surgical procedure. This failure in response often indicates that the liver is so badly damaged that such interference as a surgical procedure may easily produce hepatic insufficiency. Although the prothrombin clotting time may remain increased after vitamin K has been administered, if a fatal hemorrhagic diathesis does occur, it apparently results not from hypoprothrombinemia but from some other cause.

It is now becoming well established that deficiency of prothrombin in the circulating blood develops in cases of intestinal disorders.[246]

242. Butt, H. R., and Snell, A. M.: Unpublished data.

243. Smith, H. P., and Owen, C. A.: The Absorption of Water-Soluble Vitamin K Without the Aid of Bile Salts, J. Biol. Chem. **134**:783-784 (July) 1940. Butt and Snell.[242]

244. Scanlon, G. H.; Brinkhous, K. M.; Warner, E. D.; Smith, H. P., and Flynn, J. E.: Plasma Prothrombin and the Bleeding Tendency with Special Reference to Jaundiced Patients and Vitamin K Therapy, J. A. M. A. **112**:1898-1901 (May 13) 1939. Smith, H. P.; Ziffren, S. E.; Owen, C. A., and Hoffman, G. R.: Clinical and Experimental Studies on Vitamin K, ibid. **113**:380-383 (July 29) 1939. Warner.[228] Butt, Snell and Osterberg.[229] Rhoads.[230] Aggeler, Lucia and Goldman.[241e]

245. Butt, H. R.; Sneli, A. M., and Osterberg, A. E.: The Preoperative and Postoperative Administration of Vitamin K to Patients Having Jaundice, J. A. M. A. **113**:383-390 (July 29) 1939.

246. Albright, F., and Stewart, J. D.: Hypovitaminosis of All Fat-Soluble Vitamins Due to Steatorrhea: Report of a Case, New England J. Med. **223**:239-241 (Aug. 15) 1940. Mackie, T. T.: Vitamin K Deficiency in the Absence of Jaundice, New York State J. Med. **40**:987-995 (July 1) 1940. Weir, J. F.; Butt, H. R., and Snell, A. M.: Further Observations on the Clinical Use of Vitamin K, Am. J. Digest. Dis. **7**:485-490 (Nov.) 1940.

Apparently deficiency of prothrombin because of inadequate intestinal absorption usually developed in cases in which it was necessary to employ a limited diet or to withhold food and perform intestinal intubation in order to control an obstructive lesion. Profuse discharge from ileac stomas or severe chronic diarrhea further complicated the situation for many of these patients.

Vitamin K and the Newborn Infant.—It is generally agreed that during the first few days of an infant's life a deficiency of prothrombin exists in the circulating blood, but the cause of this deficiency is still debated. Using their own micromethod, a modification of Quick's method, Kato and Poncher [234e] reported that the prothrombin clotting time of the blood of newborn infants is for the most part high, particularly on the first day of life and that it averages forty-six and five-tenths seconds for the premature infant and forty-three and two-tenths seconds for the full term infant. A decrease in the prothrombin clotting time occurs as the infant grows older, so that by the end of the ninth or tenth day the clotting time of the full term infant averages twenty-five seconds. Similar observations were made by other investigators.[234e]

Salomonsen and Nygaard [247] reported that if extra feedings were started within two hours after delivery of the infant, subsequent development of hypoprothrombinemia could be prevented. These authors reasoned that early, extra feedings of the newborn may result in the hastening of metabolism of bacteria in the intestine, which creates a supply of vitamin K sufficient for the prevention of a relative transitory deficiency of prothrombin.

The treatment of hemorrhagic diathesis of the newborn has received considerable attention.[248] On the basis of the work presented, it appears

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247. Salomonsen, L., and Nygaard, K. K.: The Prothrombin Content in Relation to Early and Late Feedings of the Newborn: A Preliminary Report, Acta pædiat. **27**:209-218, 1939.

248. Waddell, W. W., Jr., and Guerry, D., III: The Role of Vitamin K in the Etiology, Prevention, and Treatment of Hemorrhage in the Newborn Infant, J. Pediat. **15**:802-811 (Dec.) 1939. Kuhn, R.; Wellenfels, K., and Weygand, F.: Zur Spezifität des Vitamins K, Naturwissenschaften **27**:518-519 (July 28) 1939. Nygaard, K. K.: Prophylactic and Curative Effect of Vitamin K in Hemorrhagic Disease of the Newborn (Hypoprothrombinemia Hemorrhagica Neonatorum): A Preliminary Report, Acta obst. et gynec. Scandinav. **19**:361-370, 1939. Hellman, L. M., and Shettles, L. B.: Factors Influencing Plasma Prothrombin in the Newborn Infant: Prematurity and Vitamin K, Bull Johns Hopkins Hosp. **65**: 138-141 (July) 1939. Shettles, L. B.; Delfs, E., and Hellman, L. M.: Factors Influencing Plasma Prothrombin in the Newborn Infant: II. Antepartum and Neonatal Ingestion of Vitamin K Concentrate, ibid. **65**:419-426 (Nov.) 1939. Andrus, W. DeW., and Lord, J. W., Jr.: Correction of Prothrombin Deficiencies by Means of 2-Methyl-1,4-Naphthoquinone Injected Intramuscularly, J. A. M. A. **114**:1336-1337 (April 6) 1940. MacPherson, A. I. S.; McCallum, E., and Haultain, W. F. T.: Effect of Intrapartum and Neonatal Administration of Synthetic Vitamin K Analogues on the Newborn, Brit. M. J. **1**:839-844 (May 25) 1940.

that a dose of from 1 to 5 mg. of 2-methyl-1,4-naphthoquinone, or any of the other synthetic quinone compounds mentioned, is sufficient in most instances to control hemorrhagic diseases among the newborn, and if this dose is administered at time of birth it will prevent transitory hypoprothrombinemia. One must remember that there can also be failures in treatment with substances from this group of compounds, provided sufficient hepatic damage has occurred. In the articles just referred to, the effect of the administration of vitamin K and synthetic compounds which exhibit vitamin K activity on pregnant women and the subsequent effect on the newborn were discussed. On the basis of these studies, it appeared that the same dose of vitamin K as that which is administered to newborn infants, if administered to the mother twelve to twenty-four hours prior to delivery, resulted in the baby having a higher value for prothrombin than is normally present. These synthetic compounds are apparently effective when administered either orally or parenterally, and toxic effects have not been noted on either the mother or the infant.

GENERAL FEATURES IN NUTRITION

There is little doubt that the American public, because of the press and advertising on the radio, is becoming tremendously concerned with its health. These and many other factors have undoubtedly accounted for the marked increase of self medication with vitamin preparations during the past two years. In this connection, Sebrell,[249] in a recent article, related some interesting and timely facts. He pointed out that of the $53,790,000 spent by the public for vitamin preparations during 1938, $21,000,000 was spent for products which did not conform with either the Pharmacopeia of the United States or with the National Formulary. The figure for 1935 was only about $4,700,000. Sebrell [249] expressed the belief that these figures indicate more nearly the success of the advertising campaign than the sales due to real need. No doubt people with money do purchase such preparations, but they certainly do not constitute the large group of the population which is most likely to be on a deficient diet. It was reported that three of every ten families in cities and villages do not have enough money to spend for the foods which would constitute a good, complete and adequate diet. From investigations by others, it appears that a large proportion of the diets of rural families are poorly supplied with both fruits and vegetables. There is no doubt that money solves the problem of getting enough to eat, but it does not offer assurance that the nutritional values of the diets selected will be satisfactory. It is still a good policy to obtain vitamins from the grocery store rather than from the drug store.

249. Sebrell, W. H.: Nutritional Diseases in the United States, J. A. M. A. **115**:851-854 (Sept. 7) 1940.

Although concentrated forms of vitamins may be used under many conditions, there seems little reason for using a concentrate merely because the vitamin is present in concentrated form. As Elvehjem [250] pointed out, 1 Gm. of a product containing 100 units of a vitamin may not be any more valuable than 100 Gm. of a food containing 1 unit per gram. In most cases, 100 Gm. of food would be much better since it also might contain 100 units of ten different factors.

Fortification of Food.—There are many reasons to believe that American dietaries with respect to content of many nutrient materials [251] are as a whole unsatisfactory. For these reasons a feeling has arisen during the past few years that perhaps a distinct justification for fortification of foods with vitamins and minerals exists. There seems to be no fundamental objection to the addition of synthetic vitamins to food materials, but the commercial fortification of foods must be carefully controlled.[252] Great Britain already has fortified margarine with vitamin A and restored calcium and thiamine hydrochloride to flour. Because of this the belief is expressed that ill effects from a deficiency of calcium compounds and vitamin B_1 will be minimized, notwithstanding lower rations.[253] This subject is receiving consideration by those responsible for national defense in the United States.

There are many valid objections to the fortification of foods. As Sebrell [254] pointed out, such a procedure in all probability not only would fail to be of any material value in the prevention of the diseases of deficiency because the fortified foods would not be available to the part of the population in greatest need of them, but also would be an economically wasteful procedure because it would unnecessarily increase the cost of these foods for persons whose diet is already adequate. Instead of this, he suggested that the situation might be corrected by trying to build up an association between good health and a colorful diet. This author and many others have expressed the opinion that the problem of deficiency diseases will never be solved by fortifying foods, but that it can be solved by education and by making available to every one the widest variety of natural unrefined foods at the lowest possible prices. It is amusing, as Elvehjem [250] pointed out, that many more objections

250. Elvehjem, C. A.: The Vitamin B Complex in Normal Nutrition, J. Am. Dietet. A. **16**:646-654 (Aug.-Sept.) 1940.

251. Cowgill, G. R.: The Need for the Addition of Vitamin B_1 to Staple American Foods, J. A. M. A. **113**:2146-2151 (Dec. 9) 1939.

252. Morgan, A. F.: Fortification of Foods with Vitamins and Minerals, Milbank Memorial Fund Quart. **17**:221-229 (July) 1939. Elvehjem.[250]

253. Vitamins for War, editorial, J. A. M. A. **115**:1198-1199 (Oct. 5) 1940.

254. Sebrell, W. H.: The Public Health Aspects of the Fortification of Foods with Vitamins and Minerals, Milbank Memorial Fund Quart. **17**:241-247 (July) 1939.

are raised when a commercial company wants to add a vitamin to a food than when new processing methods are introduced which are known to be destructive to the vitamin.

Effects of Cooking and Freezing on Foods.—In an extensive study, Aughey and Daniel [255] observed that destruction of thiamine amounted to as much as 22 per cent in foods boiled in water and that additional amounts up to 15 per cent dissolved in the cooking water. When the cooking water was discarded, the total losses of thiamine in vegetables frequently amounted to approximately 20 to 35 per cent. Roasting caused the loss of 43 per cent of the thiamine in pork loin. Cooking of whole grain cereals in a double boiler did not destroy the thiamine. In the light of their analysis, the authors pointed out some interesting household hints. On a serving basis, baked potatoes, including the skin, ranked higher in content of thiamine than did cooked whole wheat or oat cereal. Even boiled potatoes, spinach and carrots may be classed as furnishing an amount of thiamine comparable to those amounts supplied by cooked cereals. One slice of whole wheat bread contains approximately the same quantity of thiamine as one serving of these vegetables or cereal foods, and one serving of lean pork loin cooked either as chop or roast furnishes a plentiful daily allowance of thiamine for the adult.

Rose,[256] in an extensive study, reported that the number of microorganisms in food apparently is reduced greatly by quick freezing but that after thawing the organisms increase rapidly. For this reason it is suggested that such foods should be cooked either without thawing or soon afterward. The values of vitamin A in food are conserved by storage at low temperatures; vitamin B_1, likewise, is not affected by the freezing itself, but considerable loss may occur in the blanching of vegetables which have a tendency to lose their content of thiamine by cooking. Practically no riboflavin was lost in the quickly frozen vegetables which were investigated. The ascorbic acid in fruits also appeared to be conserved by freezing.

255. Aughey, E., and Daniel, E. P.: Effect of Cooking upon the Thiamin Content of Foods, J. Nutrition **19**:285-296 (March) 1940.

256. Rose, M. S.: The Effect of Quick Freezing on the Nutritive Values of Foods, J. A. M. A. **114**:1356-1361 (April 6) 1940.

News and Comment

The Ella Sachs Plotz Foundation for the Advancement of Scientific Investigation.—Eighty-one applications for grants were received by the trustees of the Ella Sachs Plotz Foundation during 1940. Fifty-one of these came from the United States and the other thirty from fifteen different countries in Europe, Asia, North America and South America. The total number of grants made during this year was twenty-three, one of these being a continued annual grant.

In the seventeen years of its existence the Foundation has made three hundred and ninety-four grants, which have been distributed to investigators in Arabia, Argentina, Austria, Belgium, Brazil, Canada, Chile, China, Czechoslovakia, Denmark, Egypt, Estonia, Finland, France, Germany, Great Britain, Greece, Hungary, India, Iraq, Italy, Latvia, Lebanon, Netherlands, North Africa, Norway, Palestine, Poland, Portugal, Rumania, South Africa, Sweden, Switzerland, Syria, Venezuela, Yugoslavia and the United States.

During the present great need for funds, grants will be given in the sciences closely related to medicine without reference to special fields. The maximum size of grants will usually be less than $500.

Applications for grants to be held during the year 1941-1942 must be in the hands of the executive committee before April 1941. There are no formal application blanks, but letters asking for aid must state definitely the qualifications of the investigator, an accurate description of the research, the size of the grant requested and the specific use of the money to be expended. In their requests for aid, applicants should state whether they have approached other foundations for financial assistance. It is highly desirable to include letters of recommendation from the directors of the departments in which the work is to be done. Only applications complying with the foregoing conditions will be considered.

Applications should be sent to Dr. Joseph C. Aub, Collis P. Huntington Memorial Hospital, 695 Huntington Avenue, Boston, Mass., U. S. A.

John Phillips Memorial Award for 1941.—Dr. William Christopher Stadie, associate professor of research medicine at the University of Pennsylvania, has been awarded the John Phillips Memorial Medal for 1941 for his significant contributions to the knowledge of anoxia, cyanosis and the physical chemistry of hemoglobin, and more especially for his recent studies on fat metabolism in diabetes mellitus.

This award was established by the American College of Physicians in 1929, to be given periodically for some outstanding piece of work in internal medicine, including not only clinical science but all of those subjects which have a direct bearing on the advancement of clinical science. The work must have been done in whole or in part in the United States or in Canada.

Nominations for the award are made to the Committee on Fellowships and Awards of the American College of Physicians. The recipient must file with the College a written account of his work and present his results as a paper before the next annual session, at which time the award of a bronze medal is made by the president of the College.

Annual Meeting of the American Public Health Association.—The seventieth annual meeting of the American Public Health Association will be held Oct. 14-17, 1941, in Atlantic City, N. J., in the Convention Hall. Residence headquarters will be the Hotel Traymore.

A committee responsible for entertainment, inspection trips and other local aspects of the meeting is being formed under the direction of Dr. S. L. Salasin, Health Officer of Atlantic City.

The following related organizations will meet with the American Public Health Association: the American School Health Association, the International Society of Medical Health Officers, the Association of Women in Public Health, the Conference of State Sanitary Engineers, the Conference of Municipal Public Health Engineers and the Conference of State Provincial Public Health Laboratory Directors.

The sixty-ninth annual meeting, held in Detroit in October 1940, attracted an attendance of more than 3,100 from all parts of the United States, and from Canada, Cuba and Mexico.

Book Reviews

Physical Diagnosis. By Ralph H. Major, M.D., Professor of Medicine in the University of Kansas. Second edition, revised. Price, $5. Pp. XIV + 464, with 437 illustrations. Philadelphia and London: W. B. Saunders Company, 1940.

In 1938, a year after the first edition of this book had made its appearance, a certain teacher was about to commence a course in physical diagnosis with an enthusiastic class. The students asked for a textbook which they could afford to buy in order to read, study and interline their copies. Their teacher cast about for a likely candidate. He was pleased by Major's book: by its appearance, illustrations and format; by its historical allusions; by its bibliographic references at the end of each chapter; above all, by its lucidity and charm of literary style. Not wishing to be handed a pig in a poke, he attempted to learn what others thought of it. Reviewers on the whole were complimentary. In the Far West, *California and Western Medicine* (**46**:14 [March] 1937) praised it highly. The less exuberant *Journal of the American Medical Association* (**109**:232 [July 17] 1937) and *Annals of Internal Medicine* (**11**:1916 [April] 1938) were not quite so flattering. There were a few minor faults with the book, admittedly, but on the whole these periodicals gave it favorable consideration. Naturally, that supercilious old maid from the Back Bay could be expected to do no more than turn up her nose at a newcomer. In a locality where Cabots speak only to Lowells, a textbook from the Middle West was intrusive. It could not compare with one of local breeding and, as all Boston knew, the twelfth edition of Richard Cabot's "Physical Diagnosis," with an imprint of the Massachusetts General Hospital on the title page, was about to make a bow. Thus the *New England Journal of Medicine* (**216**:726 [April 22] 1937) was aloof. Very coldly, it said that Major's book could not be recommended to students.

This particular teacher was not discouraged. He went through Major's book chapter by chapter with his class, comparing its illustrations with actual cases, making students look up references and read original articles or demonstrate on themselves or on patients the various physical signs described. In general he used the book happily as a trephine by which to drill into his pupils' heads the fundamentals of a sound knowledge of the art of physical diagnosis. The end result of this operation appeared to be eminently successful. The students not only learned but enjoyed the process.

The second edition of the book is better than the first. The author has listened wisely to advice from friends and, better still, to criticism from students. Thus the second edition is more polished than its predecessor, a better instrument for teaching and a keener-edged tool for the purpose of invading skulls a little thick. Teachers and students who still believe that a textbook on physical diagnosis is of use in medical pedagogy will welcome this edition with open arms. It warrants high recommendation.

Virus and Rickettsial Diseases: With Especial Consideration of Their Public Health Significance. Harvard School of Public Health Symposium Volume. Price, $6.50. Pp. 907. Cambridge, Mass.: Harvard University Press, 1940.

A symposium on virus and rickettsial diseases was held in June 1939 in Boston under the auspices of the Harvard School of Public Health. This volume is an amplified record of the thirty-four papers presented at that meeting. In view of the many recent developments in the field of the virus and rickettsial diseases, this volume should be welcome at the present time. It has been some years since any comprehensive work covering this field has been published in the English language.

The following virus and rickettsial diseases affecting man are discussed: variola, vaccinia, measles, mumps, dengue fever, venereal lymphogranuloma, influenza, psittacosis, poliomyelitis, epidemic encephalitis, equine encephalomyelitis, rabies, lymphocytic choriomeningitis, louping ill, yellow fever, the typhus fever group, the spotted fever group and the tsutsugamushi disease group. The review of these diseases is well carried out. The role of animals as reservoirs for certain of these diseases is considered, and such details as are known of the relation of the animal to the human disease are presented. It seems curious that inclusion blenorrhea and trachoma are not considered, whereas a lecture is devoted to a disease of animals not affecting man, distemper.

As important as the discussions of specific diseases are the papers on epidemiology, immunology, physical and chemical properties of viruses, insect vectors and clinical diagnosis and complications of the various diseases. When methods of prevention or treatment are known, these are described. One paper deals with the prevention and modification of measles, and there are two papers on the preparation and use of specific vaccines. Pertinent to the general subject matter is a paper presenting a scholarly discussion of the absorption of toxic and infectious material from the respiratory tract.

Most of the authors of the various papers are associated with Harvard University. Their writings carry the weight of authority and are straightforward and accurate. Enough historical data are given to furnish the general reader with a picture of the development of the knowledge of the different subjects.

The references listed at the end of each article are well chosen and include those which are most important.

The book has no general index, which is something of a disadvantage. The material is accessible, however, by means of the table of contents, which gives the paging of the various papers, and by means of a rather complete outline which heads each paper and gives the paging of each topic in that paper. The binding is good and the print readable.

This book does not contain detailed descriptions of the technical methods used in virus investigation and is not primarily designed for the trained laboratory worker. It is a practical and scientific presentation of the present knowledge of diseases caused by viruses and rickettsias and should be of value to public health officers and to physicians who deal with infectious diseases.

La créatine, étude physio-pathologique. By Jean Vague and Jean Dunau. Price, $1.40. Pp. 185. Paris: Masson et Cie, 1939.

In this monograph the authors have undertaken, with a high degree of success, to survey and interpret the present status of knowledge with regard to the chemistry, physiology and pathology of creatine and creatinine. Although emphasis is placed on developments in the subject since the discovery of phosphocreatine, the historical background has not been neglected, and the book is actually a review of the entire field. Preliminary chapters deal with the chemistry, determination and distribution of these compounds. A section is devoted to the relation of creatine to phylogenetic and ontogenetic development. The problem of the precursors of creatine is discussed and left as an open and important question still to be answered. In the following chapters, by means of an exposition of experimental and clinical observations, such subjects are treated as the role of creatine in the body, especially as an essential element in metabolism in muscle, and also as a possible essential in all cells, its relation to other organs, to diet and to the metabolism of carbohydrates, its storage and elimination and many other phases of its part in the organism. A picture is built up of a creatine cycle, controlled by a complex of factors, change in any one of which may lead to abnormality in the metabolism of creatine. Creatinine, as probably the sole end product of creatine, is a part of the picture. The authors state the belief that the derivation of urinary creatinine from creatine has been established and that at the present time the presence of true creatinine in the blood must be assumed. However, the anomalous behavior of the compound in blood under certain conditions remains

unexplained. The book concludes with an outline of the clinical significance which, according to the authors, may be attached to determinations of creatine and creatinine in blood and urine. Medical aspects are emphasized throughout.

This book is an interpretation as well as a review. Some of the conclusions may be questioned. For the most part a careful distinction is made between well established theories and those which are still largely hypothetic. Authorities are abundantly cited by name, and an international acquaintance with the field is manifested. The bibliography lists over 500 titles, including only publications since 1934. The reader is referred to the bibliographies of Hunter (1928) and Kayser (1934) for earlier work. A page index to authors cited in the text and a subject index (somewhat too brief) are included.

The Diagnosis and Treatment of Diseases of the Esophagus. By P. P. Vinson, M.D. Price, $4.00. Pp. 206. Springfield, Ill., and Baltimore: Charles C. Thomas, 1939.

This book meets a long-felt need for a short, concise description of diseases involving the esophagus. While several excellent books dealing with diseases of the esophagus are available, they include the entire field of pulmonary and esophageal diseases, and a great portion of such books deal with the technical phases of esophagoscopy and surgical procedures. The author has dealt with the subject in a manner that is very readable and understandable, both to the specialist and to the general practitioner. He has made a special effort to describe the various types of esophageal disease and the forms of treatment in the most simple and understandable way. He has included numerous excellent illustrations to show the various diagnostic and therapeutic procedures.

The author has had an unusual experience with the various forms of esophageal disease and has stressed the various pitfalls that may be encountered in diagnosis and treatment. While many readers may disagree with his selection of instruments for carrying out the various procedures, in the author's hands his instruments have been found eminently satisfactory. Some criticism might be made of the fact that he is prone in some instances to overlook the importance of esophagoscopy in the diagnosis of esophageal disease. He does this, however, in an effort to stress the importance of avoiding unnecessary instrumentation.

He has handled each type of disaese of the esophagus in a separate chapter, discussing the symptoms and methods of diagnosis and treatment. The chapters dealing with carcinoma of the esophagus, cicatricial stricture, cardiospasm and hysterical dysphagia are especially worthy of notice.

The last chapter is given over to a description of gastroscopy. The author has not, however, gone into the subject in any detail, and undoubtedly he does not regard gastroscopy as an essential element in the diagnosis and treatment of esophageal disease.

Endocrine Gynecology. By E. C. Hamblen, M.D. Price, $5.50. Baltimore: C. C. Thomas, Publisher, 1939.

This volume is the best that the reviewer has read on the subject of endocrine disturbances in the female. The excellent organization, clarity of style, abundance of material and generous number of unique illustrations of great merit are particularly noteworthy.

The author discusses fully the physiologic principles and properties of the hormones of the glands of internal secretion, special emphasis being placed, naturally, on the sex hormones of the pituitary gland and on the sterols secreted by the ovaries. The physiology of normal menstruation as well as bleeding, cyclic or otherwise, is covered in an extensive and thorough discussion.

The laboratory method used for diagnostic purposes in cases of endocrine disturbances is described in detail and evaluated fully. The technic of endometrial biopsy and that of tubal insufflation are set forth, and the possible information to

be derived by such procedures is extensively discussed. The various endocrinopathies found in the female and the treatment of each are described, and statements are made as to what results may be expected.

The chapters on the endocrinal aspects of functional sterility and of gestation abnormalities are commendable.

This book is replete with material that will be of value to the endocrinologist, the gynecologist, the surgeon, the general practitioner and other physicians. Throughout the volume is reflected the author's extensive knowledge of the subject and experience in dealing with gynecologic endocrinologic problems.

A full and complete bibliography adds to the value of the book.

There are both an authors' index and a subject index.

Das menschliche Knochenmark. By Karl Rohr, M.D., Private Lecturer in Medicine, University of Zurich. Price, 37 marks. Pp. 286, with 217 illustrations. Leipzig: Georg Thieme, 1940.

This is an excellent monograph. It discusses in an interesting manner everything which concerns the bone marrow: its embryology, anatomy, physiology and pathology, the character of the cells to be found in it and the varying pictures produced in it by different pathologic conditions.

The author was trained by Naegeli. Evidently he is a keen hematologist, having inherited much of his master's enthusiasm, and has enjoyed the putting together of this book. He has approached his task logically: There are first a short chapter on the medical history of the bone marrow, then a meticulous account of the technic of sternal puncture and finally chapters telling in detail the story of what properly conducted studies of bone marrow reveal.

The illustrations deserve comment, for the book is so well and attractively illustrated that the pictures alone are worth studying regardless of the text. Many of the figures are photomicrographs, beautifully clear and with excellent legends. Some are colored pictures, bringing out in diagrammatic and semi-diagrammatic form the development of the various marrow cells. Also, there are easily understandable tables and graphs appended, which demonstrate the modern hemogram and reveal how careful study of marrow cells and blood smears can be of great clinical usefulness.

Last of all is a good bibliography. It is predominantly European but gives due reference to the work of American hematologists.

On the whole, the author has compressed into a relatively short monograph a great deal of useful information. He deserves highest praise for completing a book which cannot fail to interest the general reader and yet at the same time is a useful reference work to the student or specialist whose chief occupation is to learn more of the peculiarities of the human bone marrow.

Vitamin D: Chemistry, Physiology, Pharmacology, Pathology, Experimental and Clinical Investigations. By C. I. Reed, A.M., Ph.D., Associate Professor in Physiology; H. C. Struck, M.S., Ph.D., Associate in Physiology, and I. E. Steck, M.S., M.D., Instructor in Physiology and in Medicine, Departments of Physiology and Medicine, College of Medicine, University of Illinois. Price, $4.50, cloth. Pp. 389. Chicago: University of Chicago Press, 1939.

This monograph is an excellent review and evaluation of the recent literature on vitamin D and includes, in addition, the results of numerous investigations by the authors.

The chemistry and various forms of the vitamin and the methods of chemical and biologic assay are first discussed. Subsequent chapters deal with the physiology of vitamin D and its relation to rickets and to the parathyroid glands. The influence of vitamin D on energy metabolism, mineral metabolism, resistance to infection and blood constituents is considered in detail. Two chapters are devoted to the toxic effects of large doses of various forms of this vitamin. Good results

are reported from the use of vitamin D in the treatment of arthritis, and other possible therapeutic applications are indicated. Throughout the book emphasis is placed on problems which need further investigation. When controversial subjects are discussed, tentative interpretations are offered but no final conclusions are drawn. There is an excellent and extensive bibliography, which should be extremely useful to any one interested in research in this field. The book should be especially valuable to the physiologist and to the clinician interested in diseases of nutrition and metabolism.

Bothman's Fundus Atlas. By Louis Bothman, M.D., and Reuel W. Bennett. Price, $17. Pp. 53. Chicago: Year Book Publishers, 1939.

Bothman's "Fundus Atlas" presents fifty original stereoscopic photographs of congenital anomalies and diseases of the fundus. These are mounted on cards, the photograph of the lesion being of the fundus given in duplicate accompanied with a history of the patient. There is also an excellent description of what is seen in the fundus in question.

Obviously the atlas is for the use of the ophthalmologist in teaching students, but it should be of immense service to the internist. To the medical student the ophthalmoscope has become almost as important an adjuvant to his diagnostic armamentarium as the otoscope, tongue depressor or reflex hammer. The student is taught to recognize the commoner ophthalmoscopic findings indicative of constitutional organic disease, but frequently some abnormality is noted which may be purely local. The teacher of internal medicine who is familiar with Bothman's "Fundus Atlas" can make the diagnosis and show to the student a close approximation of the lesion.

In addition to being of service to the teaching unit, the "Fundus Atlas" should be of great help to the practitioner who regularly uses the ophthalmoscope, as every practitioner should.

Pflanzliche Rohkostdiät. By Ulrich Grüninger and Hanna Gettler. Price 3.60 marks. Pp. 90. Stuttgart: Ferdinand Enke, 1939.

Diets of raw food, especially vegetables and fruit, are discussed and advised, particularly for children. A sample diet for a day is as follows: breakfast, strawberries and walnuts; lunch, radishes, salad, peas and cherries; evening meal, cucumbers, chicory salad, celery, bananas and dates. These diets are advised for various pathologic conditions, including diabetes of children, in connection with which no mention is made of insulin.

The reviewer had some difficulty in reading the old Gothic script which is used in this book.

Sexual Pathology: A Study of Derangements of the Sexual Instinct. New Revised Edition. By Magnus Hirschfeld, M.D. Price, $2.95. Pp. 368. New York: Emerson Books, Inc., 1940.

This book, written in a semipopular style, deals with aberrations of sexual instinct and behavior largely from a clinical standpoint. It is the reviewer's impression that the discussion is not based on the most profound modern psychiatric knowledge in this field, and many of the lengthy case reports could perhaps be replaced to advantage by more systematic discussions of the principles involved in these aberrations, both psychic and somatic.

Clinique et physiopathologie des maladies coeliaques. By Robert Dubois. Price, 80 francs. Pp. 350, with 14 plates. Paris: Masson et Cie, 1939.

This handsome monograph, beautifully illustrated, deals with all aspects of celiac disease, or idiopathic steatorrhea. In addition to a review of the literature the author presents his own experiences with conditions of this sort. While the pediatric side is emphasized, the book is of value to any one dealing with this group of diseases.